THE GENOMICS OF DEVELOPMENT RATE VARIATION IN *COCHLIOMYIA MACELLARIA* (DIPTERA: CALLIPHORIDAE)

by

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ABSTRACT

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Development rate is a quantitative trait that displays significant variation within many species, including *Cochliomyia macellaria* Meigen (Diptera: Calliphoridae). Calliphorids are a family of dipterans known as blow flies and are commonly used in forensic entomology to estimate the minimum postmortem interval (PMI_{MIN}), given some assumptions are made. In order to dissect the genetic underpinnings of development rate variation in this species, artificial selection for fast and slow development with population-based resequencing was used. The objective of this study is to isolate and characterize genomic regions that are correlated to development rate variation in blow flies. The first approach used known regulatory development genes from *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and isolated variants that were associated with development time changes in artificially selected fast and slow development *C. macellaria* strains. Three variants located in *Ras* and *Eip74EF* were associated with fast or slow development in selection strains with a significant change in allele frequency. The second approach involved the comparison of pooled artificially selected fast and slow *C. macellaria* genomes to investigate the genetic basis of development rate variation. When comparing the fast and slow genomes, 699 sequences were identified that contained 7290 variants with consistent changes in allele frequency. The variants indicated that the genomic regions that are associated with development rate were associated with developmental processes, including regulation of RNA polymerase II activity, and transporter activity, such as protein dimerization. Of the 699 sequences, 69 sequences were related

to *Achaete-scute complex* and 14 were related to *Cyp12A7*. The identification of the genomic regions that associated with development rates from this study provides an important resource for future studies in identifying potential genetic markers to increase the effectiveness of PMI_{MIN} estimates. By using significantly associated variants as *a priori* candidates for future studies, the data increases the understanding of natural development variation in blow flies.

1. INTRODUCTION

1.1 What is Forensic Entomology?

One of the many fields of forensic science is forensic entomology, which simply means the study of insects and their application to the court of law. Forensic entomology is broken down into three branches: medicolegal, urban, and stored products. Medicolegal forensic entomology is the use of insects to estimate some time period since death, such as the minimum postmortem interval (PMI_{MIN}), movement of the body, manner of death, and association of suspect to scene (Byrd & Castner, 2010). This information could establish a timeline of events in order to eliminate suspects or connect a body with a missing person (Catts, 1990; Geberth, 1996). Common methods used to establish a PMI are based upon predictive physical and chemical changes that a recently deceased body goes through (Henssge & Knight, 1995). As time since death increases, these methods become less reliable. The decaying body produces odors which attract both vertebrate and invertebrate scavengers to the site (Putman, 1983). The most common invertebrates to frequent carrion are commonly known as the blow flies (Diptera: Calliphoridae). Blow fly larvae feed upon dead tissue and are typically the first invertebrates to arrive at a body.

1.1.1 Two approaches for estimating PMI

Insects collected at the crime scene can be treated as entomological evidence. One method of estimating PMI_{MIN} involves inferring blow fly age based upon the species stage of development when discovered. This method estimates a PMI_{MIN} because of a set of assumptions made by the entomologist. Assumptions are made by entomologists to take into account that they do not know if the evidence contains the oldest insect, and that there are no current methods for estimating the time period between death and insect colonization. (Byrd & Castner, 2010). A second method to

estimating PMI uses succession data (Schoenly & Reid, 1987). Succession data takes into account the time between death and arthropod colonization, the appearance of particular arthropod species, and stage of decay. The PMI may be estimated with a minimum and maximum range that brackets the exact PMI, along with a separate set of assumptions when making a PMI estimate (Schoenly, 1992).

1.2 How is Forensic Entomology typically used?

While forensic entomology has many uses in criminal investigations, the common use is to estimate a PMI_{MIN} based on the blow flies collected at the scene. To begin the process of estimating PMI_{MIN}, the entomologist receives insect evidence collected at the crime scene. The next step is to correctly identify the species present on the body. Blow flies can share overlapping ranges, and not all blow fly species develop at the same rate. A study in Florida found seven different species of blow fly, including *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae), *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae), *Phormia regina* Meigen (Diptera: Calliphoridae), and *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae) (Gruner, Slone, & Capinera, 2007). Each of these fly species has their own set development rate data for the forensic entomologist to apply. Without identifying the species of blow fly, the wrong development data may be applied and skew the estimated insect age.

The next step, once the species of the blow fly has been identified, is to take measurements of the larvae and environmental conditions around the crime scene. The length of the larvae collected could be measured, or the dry weight, could be compared to preexisting development data to estimate the age of the larvae (Joseph, Mathew, Sathyan, & Vargheese, 2011). The temperature at the collection scene should be recorded for the time period before discovery. Blow flies species have temperature requirements to reach their next stage of development. Degree days

are days where the temperature is greater than the temperature development threshold for the insect, for example one degree day is when the average temperature of the day is greater than the required temperature of the insect by one degree Celsius. The accumulated degree day units are adjusted per day depending on the amount of degrees Celsius above the predetermined temperature threshold for development. Temperature and age estimation data are used to calculate PMI_{MIN}. Living specimens collected from a carrion may be raised to adulthood. This can help with species identification of the larvae. Larvae raised to adulthood could also help establish PMI_{MIN} by adding the time required for the larvae to reach adulthood to the estimated larval development time (Catts & Goff, 1992).

1.2.1 Blow fly life cycle

The process for blow flies to develop from eggs to adults is highly conserved and comprised of several stages. The first stage in the blow fly life cycle is the egg. The number of eggs a female blow fly lays can depend on the amount and quality of carrion present. *Chrysomya megacephala* females laid 71.2 ± 74.5 eggs on 10 g of media while they laid 356.2 ± 177.5 eggs on 100 g of liver (Yang & Shiao, 2012). Female blow flies lay their clutches together to increase survivor rate (Heard & Remer, 1997). The eggs hatch into first instar larvae. These larvae then go through the process of ecdysis, or molting of their cuticle, into the second instar larvae. Another round of molting produces the third instar. The third instar larvae will migrate away from the carrion when they have reached a developmental threshold ensuring they have the necessary resources to complete pupation and metamorphosis, the process of blow flies going through physical changes during development. Basic conditions, such as critical weight and minimum viable weight, can lead to variation in development time and rate. The post-feeding third instar form a puparium before undergoing pupation. Blow flies go through holometabolous development similar to beetles

and butterflies, and the fly larvae molt several times before pupating (Byrd & Castner, 2010). After metamorphosis the adult blow fly will emerge from their pupal casing and repeat the cycle.

1.3 What data types are used in Forensic Entomology?

Forensic entomologists take advantage of the developmental process of the blow fly because it is well known. Developmental data used by forensic entomologists are based on the rate of the specific blow fly species reaching each stage of development at a specific temperature. To use developmental data to estimate PMI_{MIN}, reference data with conditions similar to the environment during insect colonization must be available and usable. Forensic entomologists should use data from controlled experiments that recorded similar environmental conditions that the blow flies had experienced at the carrion or other death investigation data (Catts & Goff, 1992; Introna, Altamura, Dell, Erba, & Dattoli, 1989). To generate lab controlled development data, larval growth rates are studied under different conditions in small containers which mimic abiotic factors that the larvae may have experienced on a body. Entomologists may change factors such as temperature and humidity to mimic environmental conditions of real casework. The amount of developmental data has increased over the years, with a greater number of species and environmental factors being investigated (Byrd & Butler, 1996; Goff & Lord, 1994; Nabity, Higley, & Heng-Moss, 2006).

1.4 What contributes to development rate variation?

Phenotype variation, such as development rate, can be attributed to the interaction between environment and the genotype. Genotype-environment interactions (GEI) is the change in relative performance of genotypes in different environments. *Cochliomyia macellaria* during the summer months can be found in Texas, USA as well as Indiana, USA. These states have different environmental conditions such as humidity level and average temperature, and these factors can

influence the phenotype observed by forensic entomologists. Some of the life history traits influenced by genotype and environment include blow fly body size and development time (Mondor et al., 2012). Abiotic factors have been observed to influence life history traits such as development rate are discussed below.

1.4.1 Seasonal temperatures

The factors that influence developmental rate of different blow flies are numerous and have to be taken into account when estimating PMI_{MIN} (Byrd & Castner, 2010). A large environmental factor that is taken into consideration by forensic entomologists is the weather and seasonality of the crime scene before and during the discovery of the body and the collection of the evidence. Certain blow fly species are present when temperatures are cooler, and others when temperatures are warmer. For example *Calliphora livida* Hall (Diptera: Calliphoridae) is dominant during the cooler months while *P. regina* is present from March to October dominating during the warmer months (Nabity et al., 2006). As the seasons change blow flies may begin spreading into new territories to take advantage of more resources. One such species is *C. macellaria* that migrates north into North America during the warmer months (Whitworth, 2006). Another influence of seasonal changes is the occurrence of diapause, which is when larvae suspend development during unfavorable conditions. Insects may experience diapause in their larval or pupal stage of development in response to seasonal cues, such as decreasing photoperiod and decreasing temperatures (Gill, Goyal, & Chahil). Different blow fly species, even those closely related, show different diapause behavior. *Lucilia sericata* Meigen (Diptera: Calliphoridae) experiences diapause at both at higher and lower temperatures allowing it to spread into cooler climates while *Lucilia cuprina* Shannon (Diptera: Calliphoridae) does not diapause, which restricts its habitat to subtropical climates (Ash & Greenberg, 1975). When

observing the effect of short photoperiod on the paternal line, the offspring *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) displayed diapause behavior. *Calliphora vicina* collected from Northern Finland and Southern England exposed to the same photoperiod and temperature, L:D 15.5:8.5 at 15 °C, the offspring of Northern Finland *C. vicina* had a short day response and diapaused and the offspring of Southern England *C. vicina* had a long day response and developed into adults (McWatters & Saunders, 1997). This study supports the theory that response to the environment can be inherited, and that the geographic regions where the parents originate from also influences phenotype observed in their offspring. Weather and seasonality can influence the species of blow fly that may be available to colonize a carrion.

1.4.2 Ambient temperature

Seasonality influences the distribution of blow fly populations, but localized temperatures can influence development rate. Ambient temperature has a large and profound effect on insect development rate and metabolism (Andrewartha & Birch, 1986; Chapman, 2012). Temperatures will either decrease of increase development rate of the blow fly. *Phormia regina* raised at 30 °C developed from egg to adult in 11.8 ± 0.3 days, but when raised at 15 °C developed from egg to adult in 39.6 ± 1.8 days (Nabity et al., 2006). Colder temperatures tend to decrease development rate, while warmer temperatures increase development rate. Exposure to temperature extremes, such as greater than 35 °C, can be lethal depending on species (Bansode, More, & Zambare, 2016; Lecheta, Thyssen, & Moura, 2015). Temperature data for the time period around collection must be recorded because of the influence it may have on the development rate of the insects collected.

Sunlight and air temperature can influence the temperature of the carrion, which will then influence the blow fly larvae on the body. Forensic entomologists usually collect weather data at

the scene and/or refer to meteorological data to help them select the right set of development data to estimate the insect age and PMI_{MIN}. The location of the carrion in its surroundings could influence decay rate and the number of blow flies attracted to the volatile chemicals. For example, carrion in a shaded region attracted 26561 flies, while the carrion in the sun collected 12033 (e Castro, Sousa, Arnaldos, Gaspar, & García, 2011). The influence of temperature on developmental rate of blow flies is a well-known and studied abiotic factor.

1.4.3 Larval masses

Environmental temperatures were not the only influence on the temperature the larvae experience. Female blow flies oviposit clutches of eggs together to improve survival rate of the next generation. Larvae form larval masses on carrion for several reasons. A group of larvae can excrete digestive enzymes to help break down the carrion and make it easier for the larvae to consume the carrion (Ziffren, Heist, May, & Womack, 1953). The larval masses produce heat, typically higher than the ambient temperature, causing larval mass effect (Cianci & Sheldon, 1990; Goodbrod & Goff, 1990; Greenberg, 1991; B. Turner & Howard, 1992). The presence of a larval mass on carrion produces an Allee effect, where population size correlates with mean individual fitness (Dittmer, 1931). Allee effect is suggested to be the reason behind larval communication and aggregation on carrion (Fouche, Hedouin, & Charabidze, 2018). Extreme temperatures can occur in large larval masses which could cause an increase in larvae development, or the high temperatures could result in larvae death. Large masses may also restrict access to carrion due to overcrowding of food source surface. Larvae not in a larval mass may experience lower temperatures and a lack of digestive enzymes slowing digestion of the carrion. This can led to longer development times for the larvae, leading to an increased PMI_{MIN}

estimate. The presence of a larval mass should be noted due to their influence on developmental rate.

1.4.4 Type of carrion

Research has shown that blow flies raised on different organs may also develop at different rates (Boatright & Tomberlin, 2010). The carrion that the larvae are feeding on can also influence the development rate of the blow fly. Blow fly larvae can develop on a variety of carrion types, including invertebrate and vertebrate tissue, as well as excrement and other types of organic matter, albeit this tends to be species specific. Different tissues have shown to interact with time to affect larval weight (Boatright & Tomberlin, 2010). For example, larvae were heavier and grew faster when grown on canine tissue vs. those raised on other tissue (equine, porcine) at 28.3 °C. This indicates that with the same temperature, the carrion type can influence development rate. Blow fly larvae have even been found growing in the feces of a dirty diaper, presenting another scenario where the media the larvae developed on needs to be investigated for its influence on the development rate (Goff, Charbonneau, & Sullivan, 1991).

1.4.5 Toxins present in carrion

Different contaminates, such as drugs, in the carrion itself can influence the development rate of blow fly larvae, which can then influence PMI_{MIN} (Goff & Lord, 1994). Without performing a toxicology exam on the carrion, the possible influence of drugs may not be accounted for when estimating PMI_{MIN}. The development rate may be increased or decreased depending on the substance present and its concentration in the body. When exposing *Chrysomya albiceps* Wiedemann (Diptera: Calliphoridae) and *Chrysomya putoria* Wiedemann (Diptera: Calliphoridae) to livers containing cocaine, the blow flies reached adulthood 60 hours before the blow flies exposed to control liver (de Carvalho, Linhares, & Palhares, 2012).

Meanwhile, a study observed that the drug Buscopan®, a compound used to treat gastrointestinal disorders, decreased development rate (Oliveira, Gomes, Morlin Jr, Von Zuben, & Linhares, 2009). Entomologists may want to request a toxicology report if evidence indicates drugs may be involved.

1.4.6 Blow fly species present on carrion

Another factor that can influence the development rate of larvae collected at a crime scene is the species of larvae collected and how those eggs, or larvae, are laid on the carrion. The identification of the species collected is important for several reasons. Different blow flies species can take different amounts of time to detect and arrive at the body. Typically blow flies oviposit eggs around orifices or wounds on the body, but certain species such as *Calliphora varifrons* Malloch (Diptera: Calliphoridae) lay a mix of first instar larvae and eggs on the carrion (Cook, Voss, & Dadour, 2012). Ovoviviparous is where the eggs hatch inside the female blow fly allowing for live larvae to be deposited on carrion. Myiasis is the exception to this claim and has to be considered by forensic entomologists when present at the crime scene because of the influence they would have on estimating the PMI_{MIN}. It is the infestation of living vertebrates by blow fly larvae, but this is a rare occurrence in North America (James, 1947; Zumpt, 1965). One common blow fly that causes myiasis is the *Cochliomyia hominivorax* Coquerel (Diptera: Calliphoridae). These blow flies can be observed not only in death investigations, but in criminal abuse and neglect cases (Thyssen, Nassu, Costella, & Costella, 2012). *Cochliomyia hominivorax* has been eradicated from North America and most of Central America since the 1980s using sterile insect technique (Krafsur, Whitten, & Novy, 1987; Novy, 1991).

1.4.7 Predation behavior of certain blow fly larvae

Multiple fly species may colonize the same food source which can lead to predation of one fly species on another, such as *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) cannibalizing *Chrysomya megacephala* Fabricius (Diptera: Calliphoridae) (Goodbrod & Goff, 1990) and *C. macellaria* (Faria, Orsi, Trinca, & Godoy, 1999; Wells & Greenberg, 1992). The influence of one species cannibalizing another can cause the prey species to increase development rate to avoid competition for resources (Brundage, Benbow, & Tomberlin, 2014; Wells & Greenberg, 1992). Predator species receive fitness benefits by colonizing the same carrion after the prey species allowing the larvae to eat both carrion and prey (Brundage et al., 2014). Interaction of blow fly species may adjust the overall development time of the larvae, which then influences PMI_{MIN} estimates.

1.5 What about the genetic contribution to development rate variation?

While there has been a lot of work done to study the environmental effects on development rate, not a lot of work has been done to study the influence of genetics. A few studies have used "common garden experiments" to explore development rate variation between the same fly species from different geographic regions. These experiments involve taking subjects from multiple geographic locations and raising their offspring in the same environment. Variation in development of blow fly offspring raised in the same abiotic environment can be linked to genetic differences present in the geographic regions of the parent blow fly. One researcher tested a variety of environmental factors on the offspring of *L. sericata* collected in East Lansing, MI. The development results from *L. sericata* raised under the same temperature and humidity while the substrates produced a range of development times from 329 to 505.5 hr, similar to the range of development time in published studies (A. M. Tarone & Foran, 2006). Another studies

used *L. sericata* strains from three different geographic regions, California, Michigan, and West Virginia, and raised at two different temperatures, 20 °C and 33.5 °C. The size of the pupae and development time were significantly different between the geographic strains and temperature, indicating that genetic differences and variation in environmental response influence development rate (A. M. Tarone, Picard, Spiegelman, & Foran, 2011). The same species of fly, *L. sericata*, was collected from Sacramento, CA, San Diego, CA, and Boston, MA and raised under three different temperatures, 16 °C, 26 °C and 36 °C. The offspring were raised at 16 °C both Sacramento, CA and Boston, MA developed 23 to 25 hours faster than San Diego, CA. The faster developing blow flies originated from Sacramento, CA and San Diego, CA by developing 25 hour faster than Boston, MA at 26 °C. At 36 °C, blow flies originating from Sacramento, CA and Boston, MA developed eight to ten hours faster than blow flies originating from San Diego, CA (Gallagher, Sandhu, & Kimsey, 2010). Using developmental data established using *L. sericata* from a different geographic region can lead to an increase in PMI_{MIN} error, up to 13.8%. This indicates that blow flies from different states in the United States of America, with different abiotic conditions, show development rate variation when the offspring are raised in the same abiotic conditions. An investigation using *C. macellaria* from three distinct geographic regions in Texas saw development variation when raising the blow flies at 21 °C 65% RH (C. Owings, 2014). The larval development took nine hours longer for blow flies originally from Longview, TX when compared to those originally from College Station, TX. Blow flies originally collected from Longview, TX had a pupal development period that was 29 hr shorter than flies collected from College Station, TX. This study shows that blow flies from the same species and located from different regions in the state and raised under the same environmental conditions in the lab display variation in development rate. These experiments give evidence that the genetics of the

blow flies do play a role in the development of the blow fly since development rate variation is observed when environmental conditions are controlled.

1.6 How can the study determine what genetic components influence development rate?

There are several methods to investigate the influence of genotype on phenotype. One method is association mapping, also known as linkage dissociation mapping, which maps quantitative trait loci using historic linkage disequilibrium. Common garden experiments, also known as transplant experiments, take organisms from different geographic regions and raise the offspring in the same environment to observe if there is a genetic and/or environment component to the phenotype in question. Gene expression experiments measures the activity of genes creating the picture of cellular function. The expression levels can be compared between treatments, such as the expression level of genes associated with development rate in fast and slow developing blow flies, to associate genes that are over or under expressed with a phenotype. The method used in this study is population-based resequencing of strains that have been artificial selected for the phenotype, called evolve and resequence (E&R) (Thomas L. Turner, Stewart, Fields, Rice, & Tarone, 2011). In E&R studies, diverging strains are formed from applying pressures of selection for a number of generations. Once a significant divergence in the phenotype is observed, then pooled DNA can be sequenced using next-generation methods. The pooled genomes create a snapshot of the composition of the population's DNA. From the pooled genomes of the selected strains, loci can be isolated and characterized to discover loci that are either associated with the genetic contributor, or the actual cause of genetic changes associated with the phenotype.

1.6.1 Pooled sequencing method

Technology has advanced producing new tools and methods to identify and characterize genotype-phenotype associations. Next-generation sequencing (NGS) technology allows for sequencing of whole genomes or selected regions of DNA. Next-Generation Sequencing can be used to sequence pools of DNA from many different individuals called pool-seq. Pool-seq is a cost effective and time efficient method that can be used instead of sequencing many individuals separately, yet still produce similar coverage. The resulting data from pool-seq can then be compared to each other using the field of comparative genomics.

Most foundational studies of E&R have been done using *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). For example, extensive selection experiments have been done to investigate the genetic contributions to body size variation (Thomas L. Turner et al., 2011), courtship song variation (T. L. Turner & Miller, 2012), accelerated development (Burke et al., 2010), hypoxia tolerance (Zhou et al., 2011), lifespan and late-age fertility (Remolina, Chang, Leips, Nuzhdin, & Hughes, 2012), adaptation to novel environments in elevated temperature regime with daily temperature fluctuations from 18 °C to 28 °C (Orozco-terWengel et al., 2012), microRNA control of genomic diversity (Cassidy et al., 2013), response to hot or cold environments (Tobler et al., 2014), diet (Reed et al., 2014), and egg-size (Jha et al., 2015). Results from these studies linked phenotype to changes in genotype. The results from these studies can help frame artificial studies of other insects, such as the selection study performed using *C. macellaria* in Texas to isolate and characterize changes in genotype as they relate to a phenotype (Aaron M Tarone, Picard, & Sze, 2016).

1.7 Developmental process and involved genes

1.7.1 Hormones and development

Genes linked to developmental rate regulation and timing are well known in *Drosophila* in relation to the process of metamorphosis. The hormonal system is most associated with the process controlling metamorphosis (McBrayer et al., 2007; Yamanaka, Rewitz, & O'Connor, 2013). The titers of hormones during transition periods between the stages of *D. melanogaster* development dictate when the next step of the life cycle begins (Dubrovsky, Dubrovskaya, & Berger, 2004). These titers reflect the two main hormones involved in the life cycle of the blow fly, juvenile hormone (JH) and the ecdysone hormone. Juvenile hormone controls predominantly the transitions between instars and ecdysone controls the transition from post-feeding third instar to adult through pupation (Yamanaka et al., 2013). The process of initiating metamorphosis using hormones takes several pathways working together to ensure the blow fly instar is ready to make the journey from pupariation to adulthood.

1.7.2 Nutrient signaling pathways

Many factors come together to start the post feeding third instar on its journey to adulthood. These factors include critical body weight, photoperiod (light/dark cycle), signaling cooperation, and nitric oxide (Yamanaka et al., 2013). Critical body weight is the body size achieved by the instar to initiate metamorphosis where they will not starve during pupation (NIJHOUT & WILLIAMS, 1974a, 1974b). Once critical weight has been reached, the instar can then leave its food source and find a location to proceed to pupation. Nutrition levels and critical weight ties into the regulation of ecdysone production through two different pathways. Insulin-signaling pathway helps monitor the amount of insulin being produced in relation to the amount of food being consumed. Another pathway that ties into ecdysteroidogenesis is the TOR pathway which works

with connecting nutrition and development. The TOR pathway also works hand and hand with the MAPK pathway by sharing common targets. Nutrition does play a large role in the regulation and timing of metamorphosis, but it is only part of the system that regulates ecdysone production.

1.7.3 Prothoracicotropic hormone signaling pathways

The MAPK signaling pathway works with regulating developmental timing with the prothoracicotropic hormone (PTTH). Prothoracicotropic hormone is produced by two neurosecretory cells in the brain when signaled by environmental cues such as the photoperiod and biological cues such as the JH (McBrayer et al., 2007; Sakurai, 1984). It used to be considered that PTTH was the only cue required for the production of ecdysone, therefore without PTTH the third stage instar would not proceed through metamorphosis, but after a loss of function study the post feeding third star continued through metamorphosis (McBrayer et al., 2007). Torso is a receptor of PTTH which works with the MAPK pathway to upregulates enzymes that are involved with ecdysone production which stimulates the prothoracic gland (PG) (McBrayer et al., 2007). The MAPK pathway and the TOR pathway work together to target the same enzymes to upregulate ecdysone production.

1.7.4 Activin signaling pathway

Another pathway involved in the regulation of ecdysone production is the Activin signaling pathway (Ying Y Gibbens, James T Warren, Lawrence I Gilbert, & Michael B O'Connor, 2011). This pathway works with both the MAPK pathway and the insulin signaling pathway by upregulating *torso* and *InR* within the PG cells. This pathway is important because it gives the PG cell the ability to detect and respond to developmental (PTTH) and nutritional (insulin) signals. This pathway also helps the two different signals coordinate together in the same cell. Without this pathway the ecdysone production may start too early or late due to a lowered ability to detect these

signals, or one signal is detected before the other and metamorphosis starts before the instar is ready. This is one reason multiple pathways regulate development: to prevent the organism moving ahead in development before it is ready.

1.7.5 Nitric oxide pathway

The fifth pathway involved in the regulation of metamorphosis is the nitric oxide (NO) pathway (Bialecki, Shilton, Fichtenberg, Segraves, & Thummel, 2002; Parvy et al., 2005). Several insects detect the oxygen level in the environment as a key signal in their development timing determination and body size (Callier & Nijhout, 2011; Kaiser et al., 2007). Certain nuclear receptors (E75 and βFTZ-F1) that are a part of the NO sensing pathway are actually found in the PG cell and are required for proper ecdysteroidogenesis to take place. The NO binds to receptor E75 to induce the expression of βFTZ-F1, which then upregulates the production of ecdysone (Cáceres et al., 2011). Not much is known about the use of NO as a messenger, it does seem to play a role in developmental timing as seen in other insect models. This is the fifth of five signaling pathways involved in the regulation of ecdysone production in PG cells which then controls the timing of metamorphosis.

1.7.6 Two approaches for identifying variants between genomes

There are several approaches that can be used to compare genomes against each other and to isolate regions with significantly divergent loci. One method that can be used takes reads from different DNA pooled genomes and aligns them to the same reference. This allows for analysis to compare the sequencing results at the same positions across different genomes. Variants can then be called by comparing the reference sequence to the pooled genome sequences. A second approach is to use tools specific for comparative genomics. The second approach was used for measuring differentiation between populations, genome wide association studies, and for

experimental evolution/artificial selection strains (Kofler, Pandey, & Schlotterer, 2011). A specific toolkit was designed for analysis of pooled genomes and locating significantly diverged SNPs based on allele frequencies between populations and between replicates. This toolkit goes beyond the calling of divergent variants and providing their frequency. When comparing two pooled genomes from artificial selection studies it is important to discover which loci are statistically significant between the two pooled genomes. The statistically significant variants that have diverged in allele frequency between the two artificially selected genomes can then be characterized for similar genes to discover what biological and molecular processes they may be involved in.

1.8 The blow fly of interest and related model organism

The blow fly focused on is *C. macellaria*, known as the secondary screwworm. *C. macellaria* stands out from other blow flies in the United States because of its orange head, metallic green coloring, and three black stipes on its thorax. This particular species of blow fly lives in regions with temperatures ranges around 15 °C to 39 °C and relative humidity ranges around 45% to 67% (C. Owings, 2014). During the summer months, the fly populations spread north to southern Canada from the tropical and subtropical environments of the Western Hemisphere (Novy, 1991). Like most species of blow flies, they only colonize dead flesh and are attracted to their food source by the volatile chemicals given off as the flesh is broken down by bacteria. Female blow flies require protein to develop their ovaries and they oviposit on dead flesh so their instars have a food supply available when they emerge. The secondary screwworm was chosen for this project because it is forensically relevant, cousin to the primary screwworm (a major pest species), and found through a large portion of the United States.

1.9 What does this study plan to accomplish?

This study's main goal is to apply two approaches to isolate and characterize genomic regions that influence development rate variation. The first approach to accomplish this goal is to use known development genes from *D. melanogaster* to search for homologous genes in *C. macellaria.* Using bulked segregate analysis, variants that correlate with fast or slow development may be identified within the known genes and be applied as genetic markers for development rate. The second approach is to compare artificially selected fast and slow developing *C. macellaria* strains to isolate the genomic regions that contain significant variants that experienced change in allele frequency with change in phenotype. The sequences containing the variants will then be characterized to discover which biological processes and molecular functions effect development rate variation. By identifying significant variants in the *C. macellaria*, the data should provide an understanding of the biological processes that affect natural development rate variation in blow flies.

2. DEVELOPMENT RATE GENETIC MARKERS

2.1 Introduction

Development rate is an important phenotype. For arthropods, the controlling element of developmental timing and rate is typically a result of their hormone cycle. Juvenile hormone and 20-hydroxyecdysone (the activated form of ecdysone) titers initiate the transition to the next stage of development. In *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), juvenile hormone levels increase when molting through the different instars, while ecdysone titers increase during metamorphosis (Yamanaka et al., 2013). Ecdysone binds to the nuclear receptor complex Ecdysone receptor/ultraspiracle (EcR/USP) which controls gene expression throughout metamorphosis (Carl S Thummel, 1996). Regulatory components of ecdysone production are tied into several pathways that monitor environmental and organismal conditions (Yamanaka et al., 2013). The pathways that regulate metamorphosis include mitogen-activated protein kinase (MAPK) signaling, Insulin signaling, TOR signaling, NO signaling, and Acitivn signaling. Changes within these signaling pathways, such as blocking Activin signaling, have been shown to slow down, or even stop, an organism from going through metamorphosis (Ying Y Gibbens et al., 2011). Changes in the genetic components of these signaling pathways influence the development rate phenotype of *D. melanogaster* (Ying Y. Gibbens, James T. Warren, Lawrence I. Gilbert, & Michael B. O'Connor, 2011; Layalle, Arquier, & Léopold, 2008; Yamanaka et al., 2013).

To study the variation of such a phenotype (development rate), and potentially locate genes that influence the variation, artificial selection experiments have been used (Orozco-terWengel et al., 2012; T. L. Turner & Miller, 2012; Thomas L. Turner et al., 2011; Zhou et al., 2011). A common model system used to study genotype-phenotype correlations is *D. melanogaster*.

Artificial selection studies have used an experimental design called E&R, population-based resequencing of artificially developed population, to locate genetic changes between the artificially selected strains (Burke et al., 2010; Nuzhdin, Harshman, Zhou, & Harmon, 2007; Zhou et al., 2011). The first few studies looked at body size variation (Thomas L. Turner et al., 2011), courtship songs (T. L. Turner & Miller, 2012), and longevity (Nuzhdin, Pasyukova, Dilda, Zeng, & Mackay, 1997). Body size variation in *D. melanogaster* was associated with 1633 variants in 632 genes, most of which involved in post-embryonic development, metamorphosis, and cell morphogenesis. They inferred that the timing of metamorphosis is likely to alter adult size, as well cell size and shape. When selecting for courtship songs, more than 13,000 variants were identified spread throughout the *D. melanogaster* genome, making it difficult to locate which genes the selection for courtship song effected strongly. When investigating genetic links to longevity in *D. melanogaster*, five quantitative trait loci were identified that were related to age-specific effects on survivorship and mortality. The E&R approach to identify genetic variants that are responsible to phenotype variation can indicate the genomic regions that respond to selection.

For our purposes, we are interested in development rate variation because that is the phenotype that is most important in forensic entomological investigations. Plenty of laboratory based studies exist on the effect of various abiotic and environmental factors (Boatright & Tomberlin, 2010; Byrd & Butler, 1996; Goodbrod & Goff, 1990), but little has been done to determine the genetic component (Cyr, 1993; Gallagher et al., 2010; A. a. F. Tarone, David, 2006). Therefore, the goal of this study was to determine the link between regulatory developmental genes and developmental rate variation in the forensically relevant blow fly *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) (Boatright & Tomberlin, 2010; Byrd & Castner, 2010; C. G. Owings, Spiegelman, Tarone, & Tomberlin, 2014). The aim was to discover the underlying genetic

mechanisms responsible for developmental phenotypes that could influence minimum postmortem interval (PMI_{MIN}) estimates.

2.2 Materials and Methods

2.2.1 Identifying variants potentially associated with development rate

Genes of interest were extracted from FlyBase using the following gene ontology (GO) terms: development rate, positive development rate, heterochrony, development rate control, metamorphosis, ecdysone, and ecdysteroidogenesis (Gramates et al., 2017). Fifty seven genes were then searched against the *C. macellaria* fast and slow selection strain genomes and alignment quality and variant presence in both sets of genomes ('fast" and 'slow' selected genomes from two geographic areas, College Station and Longview) was used a criteria for further analyses resulting in the selection of ten genes: *Smad2, Tsc1, Ras, Eip74EF, rin, Itgbn, EcR, Raptor, InR,* and *Babo* (Table 1). For a *C. macellaria* sequence to be identified as a putative homolog, E-value cutoff of 1E-10 was used (Morgulis et al., 2008). Amino acid sequences were predicted from the resulting sequences for the ten genes using Augustus (Stanke, Steinkamp, Waack, & Morgenstern, 2004). The multiple sequence alignment tool Clustal Omega was used to align the amino acid and nucleotide sequences between the two selected strains and the baseline strain (McWilliam et al., 2013). Locations of variation, such as indels (insertion or deletions of nucleotides) and SNPs, between the fast and slow strains were recorded for nucleotide (and resulting amino acid substitutions, if present). Within the ten genes 29 variants were identified that appeared to be associated with the phenotype: 28 SNPs and one insertion.

Starting allele frequencies (in the pooled-seq data) were noted via read mapping done in CLC Genomics Workbench v9.0.1 (https://www.qiagenbioinformatics.com/, QIAGEN Inc.,

Aarhus, Denmark). The variants with a change in allele frequency between the fast and slow genomes equal to or greater than 0.5 were considered further considered. To determine whether the variants were a false positive (a result of sequencing variation), primers were designed in conserved flanking regions with Primer3web v4.1.0 using default parameters to sequence in additional specimens (Koressaar & Remm, 2007; Untergasser et al., 2012).

Table 1. *Drosophila melanogaster* genes and their associated involvement in development rate.

Gene	Biological Process
Babo	Type I receptor for activin-like ligands; transcriptional regulation of activin responsive genes (Brummel et al., 1999)
EcR	Ecdysone receptor; molting, metamorphosis with ultraspiracle protein to form the ecdysone receptor (Davis, Carney, Robertson, & Bender, 2005)
Eip74EF	Ecdysone induced protein; transcription factor, associated with puparium formation, autophagy (C. S. Thummel, 2001)
InR	Insulin transmembrane receptor; influences total development time (Shingleton, Das, Vinicius, & Stern, 2005)
Itgbn	Integrin; cell adhesion, cohesion (Caldwell, Walkiewicz, & Stern, 2005; Hynes & Zhao, 2000)
Ras	Within GTPase family; cell division, growth pathways (Caldwell et al., 2005)
Raptor	Regulation of growth, eclosion from puparium (Foster et al., 2010; Kim et al., 2002)
rin	Ras protein signal transduction, positive regulation of gene expression (Pazman, Mayes, Fanto, Haynes, & Mlodzik, 2000)
Smad2	Activates downstream gene transcription outside of cell signals (Brummel et al., 1999; Massagué & Chen, 2000)
Tsc1	Suppresses cell growth; TOR signaling pathway (Miloloza et al., 2000)

Table 2. Primers used for sequencing *C. macellaria* from fast and slow selected strains to test the prediction panel. Primers were also used to sequence *C. macellaria* wild type offspring. Tm, melting temperature, is the temperature in which DNA is half denatured.

Primer	Sequence (5'->3')	Amplicon length (bp)	Tm (°C)
Raptor.F	GTTGAACGTGAACTGCGTGT	548	56.2
Raptor.R	ACGGCCACCGTATTGTCTAC		57.0
Itgbn.F	TGGAAAGAAGAGGCACGTAAA	491	54.2
Itgbn.R	AAGAGGGAAATTGACCACCA		54.0
EcR.F	TTGAGGATCTGCTGCATTTCT	433	54.4
EcR.R	GCCGTTGTTATACCCGATGT		55.0
rin.F	TTCTGCCACAACTGGTTTGA	489	54.9
rin.R	TGCTGTTGTTTAGCAGCTTCA		55.2
Eip74EF.F	CAACAGTCGGTTCGACCAGT	422	57.3
Eip74EF.R	CGCCAATATGCCTCTTTGAT		53.2
InR.F	TATAGGGCAACCGGCTTCAT	508	56.2
InR.R	GTTGGGATTTGAACTCATCG		51.5
Babo.F	CCCACCTGCATGGTAAAAAT	481	53.4
Babo.R	GTTTCCCGGAACCAAGAAG		53.6
Smad2.F	CCGCTTTTTGGTGTTCCATA	328	53.5
Smad2.R	CAACCGGGTGGTATTTTACA		52.7
Tsc1.F	CTACAACGCCCTTGTCGAAT	615	55.2
Tsc1.R	CCGAACGATAATCCTTGCTC		53.3
Ras.F	CCCAAGCCACAGCTGTCTA	486	57.2
Ras.R	CCAATTGTGCGGAATGAGTA		53.0

2.2.1.1 Amplification and sequencing of developmental genes

To test the hypothesis that there was an accumulation of nucleotide changes in the ten genes outlined above following selection, 24 flies were chosen from the selection experiment (but not a part of the original pooled DNA from the sequencing experiment). These included four flies each from: Longview "fast", "slow" and "baseline" and four flies each from: College Station "fast",

"slow" and "baseline." DNA extraction from the heads of these flies followed standard DNA organic extraction protocol, eluting the DNA in 60 µ TE.

2.2.1.2 DNA amplification

PCR reactions for each gene consisted of 7.5 µL 2x PCR master mix (Thermo Scientific™, Watham, MA, USA), 4.5 µL sterile water, 0.67 µM of each primer, and 1.0 µL template DNA, for a final volume 15 µL. Each PCR reaction was amplified using a Mastercycler® pro thermal cycler (Eppendorf, Hamburg, Germany) using the following conditions: 94 °C for 30 sec, touchdown from 61 °C to 51 °C over nine cycles for 30 sec, followed by 35 cycles at 51 °C for 30 sec, then one minute at 72 °C. To determine amplification quality, 5 µL PCR product was run on a 1% agarose gel (100 volts for 30 min). Successfully amplified PCR products were purified by either ExoSAP-IT (Thermo Fisher™, Foster City, CA, USA, according to manufacturer's protocols) or a QIAquick PCR purification kit (QIAGEN Inc., Valencia, CA, USA, according to manufacturer's protocols).

2.2.1.3 Sequencing amplification and genotyping

Sequencing was done using BigDye® Terminator v3.1 Cycle Sequencing Kit Ready Reaction Mix (Thermo Fisher™, according to manufacturer's protocols) at a ¼ reaction ratio using the forward primers (except *InR* was sequenced in both directions). Sequencing products were purified using BigDye® Xterminator™ clean-up kit (Thermo Fisher™, according to manufacturer's protocols). For sequencing separation and detection, 1 µL of the purified sequencing reactions were added to 10 µL of Hi-Di™ Formamide (Thermo Fisher™), and

separated and detected on a 3500 Genetic Analyzer (Thermo Fisher™) using standard sequencing protocols.

Sequences were manually edited using Seq Scanner 2.0 (Thermo Fisher™). Peaks which were poorly resolved on either the 5' or 3' ends were removed, and any located within well resolved sequences were labeled as "N." Directly overlapping peaks with the similar height were labeled has heterozygotes. The sequence editor BioEdit v7.2.5 (North Carolina State University, Raleigh, NC, USA) was used to align the sequences for each gene (including the selection experiment empirically determined sequences) (Hall, 1999). Changes in allele frequencies (between the 'fast' and 'slow') for each of the 29 identified variants were calculated.

2.2.2 Wild fly sequencing

2.2.2.1 Preparing replicates from wild *C. macellaria*

Wild flies were collected and used for a laboratory development study in which the slowest and fastest developers from a given sample were sequenced at the above loci. *Cochliomyia macellaria* were collected from Military Park, Indianapolis, IN, USA (39.7706 °N 86.1687 °W) on three separate weeks to found each replicate colony: four times between July 31st 2017 – August 4th 2017, four times between August 7th 2017 – August 14th 2017, and two times between August 19th 2017 – August 20th 2017. On average 100 *C. macellaria* were used to form each replicate and placed into a cage in a Percival incubator (Percival Scientific, Inc., Perry, IA, USA) set at 29 °C, 70% relative humidity (RH), and 12:12 L:D (light:dark). *Cochliomyia macellaria* flies were given sugar and water *ad libitum.*

2.2.2.2 Development of wild offspring

For each colony prior to the development study, chicken liver was provided to encourage reproductive organ development in females. After a few days, chicken liver was provided (<4 hours) for egg collection. For this experiment, there were two types of replicates (three biological replicates from the three different colonies founded) and for each biological replicate, between six and 18 replicate jars. Each jar consisted of approximately 100 eggs per 50 g of chicken liver. Each replicate was placed in a glass mason jar (946.35 mL, Ball®, Broomfield, CO, USA) with wood chip bedding and set in an incubator at 28 °C, 60% RH, and 12:12 L:D. Replicates were checked twice daily, and once adult flies began to emerge, jars were checked every three hours from the start of the light cycle until the end of the light cycle. Adult flies were flash-frozen for 5 min at -20 °C and stored in Falcon™ tubes (10 mL and 50 mL, Corning Inc., Corning, NY, USA) with 70% ethanol at -20 °C. Development time was calculated from the start of the egging period to the end of the three hour adult collection window.

2.2.2.3 DNA extraction

Both males and females were selected in approximately 50:50 ratio which masked any potential sex differences. Ten female and 10 male flies that emerged first (fast developers) and last (Slow developers) were separated from the rest of the adults (N = 120 total, or 40 flies from each biological replicate). Blow flies selected for DNA extraction were randomly selected from the separated fast and slow developers, with 10 fast developers selected and 10 slow developers selected. The 20 selected flies had their heads removed with flame sterilized forceps and placed into a clean 1.5 mL centrifuge tube for DNA extraction, done as above, eluting in 60 µL TE buffer and stored at -20 °C. Extracts were amplified and sequenced using the same procedures outlined above.

2.3 Results

2.3.1 Selection of genes

Gene ontological selection resulted in 10 genes selected for further analysis. Among these ten genes, which had high quality alignments, 29 variants were isolated in well conserved regions, 28 SNPs and one insertion (Table 1). For each gene, changes in allele frequency (based on read mapping) resulted in six variants located in five genes with changes > 0.5 between the fast and slow: *Smad2, Ras, Eip74EF, EcR*, and *Babo*. The variants in *Smad2, Ras, EcR*, and *Eip74EF* were located in predicted coding regions, whereas the variant in *Babo* was predicted to be located in the intron for the fast genome and in an exon in the slow genome. The difference between the fast and slow sequences could be due to the prediction tool using a probabilistic model to estimate the most likely gene structure since the actual structure of *Babo* in *C. macellaria* is not known.

Selection Strain Sequencing

To eliminate sequencing errors contributing to allele frequency differences, DNA sequencing was done on additional flies not used in the selection experiment and subsequent sequencing data (Table 3). The gene *InR* did not sequence beyond the location of the insertion in both directions, therefore no variant frequency was calculated. Three variants of the six identified from comparing the fast and slow genomes, two in *Ras* and one in *Eip74EF*, verified a change in allele frequency greater than 0.5 (Table 3). The remaining three variants had allele frequency changes between 0.16-0.33, albeit in the same direction as the fast and slow genome comparison change in allele frequency. When observing the association between genotype and development rate, Smad2 at position 1688 and Ras at position 956 had distinct changes in the genotype frequencies (Figure 2).

2.3.2 Wild offspring development and sequencing

Three replicate growth studies were done using *C. macellaria* collected from Military Park with a total of 36 jars. The majority (87.5%) of *C. macellaria* emerged between 225 hr to 275 hr. Fast developers made up 5.3% of the wild offspring that emerged before 225 hr and the slow developers accounted for 7.2% of the wild offspring emerged after 275 hr (Figure 1). From the 10 sequences generated per development rate, eight to nine sequences on average produced high quality sequencing products. Of the six variants associated with development rate, zero had changes in allele frequency were equal to or greater than 0.5 in the wild offspring. When observing the trend of allele frequencies associated with development rate, the wild offspring allele frequencies were similar to the slow selection strain allele frequencies at the variant sites Ras956 and Eip74EF467, whereas sites Smad21688, EcR1066, Ras980, and Babo505 had wild offspring allele frequencies that resembled the fast selection strain allele frequencies (Table 3). Wild offspring alternate allele frequency at variant site Ras980 was greater in the wild offspring slow developers that the fast developers, whereas the alternate allele frequency was greater in the fast developing selection strain. The wild offspring change in allele frequency, 0.35, for Smad21688 was similar to the change in allele frequency from the fast and slow genomes, 0.5, but in the selection strain sequencing the change in allele frequency was only 0.16 (Table 3). No genotypes were associated with fast or slow development, except for Smad2 at position 1688 which present GT being associated with fast development and GG being associated with slow development (Figure 3).

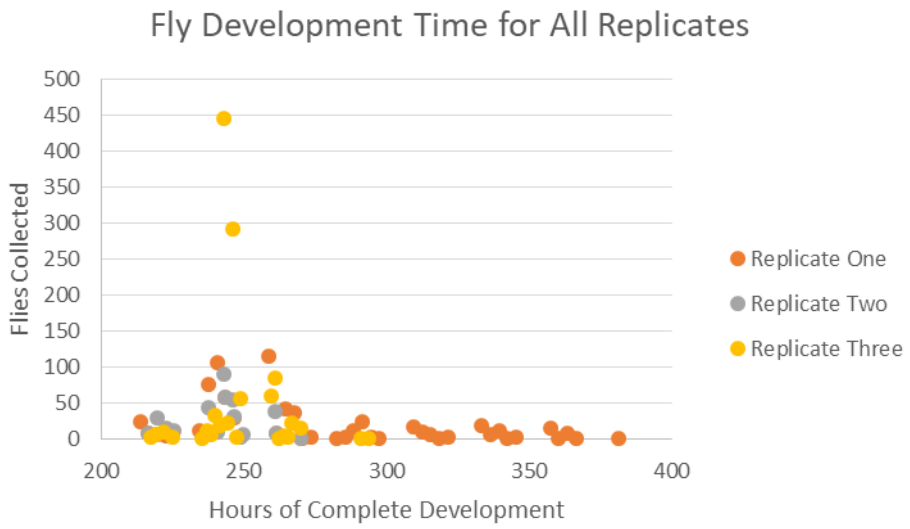

Figure 1. Complete development time from egg to adult of the flies collected from all three replicates. On the y axis is the amount of flies collected as adults at they emerged as adults. The x axis is the number of hours from oviposition to emergence.

Table 3. Prediction panel and sequencing results from the selection strain sequencing and the wild type offspring sequencing of *C. macellaria*. The position of the locus is from the location within the reference consensus sequence. Column A is the alternate allele frequencies and the change in alternate allele frequency from comparing the fast and slow genomes. Column B is the results of the fast and slow selection *C. macellaria*, including the alternate allele frequencies as well as the change in alternate allele frequency. Column C is the results from sequencing extreme fast and slow developers of wild offspring *C. macellaria*, including alternate allele frequencies and the change in allele frequency. Pos is the position in the contig of the nucleotide. Ref is the allele in the reference sequence. Alt is the variant found when comparing the fast and slow genomes. Alt AF is the frequency of the alternate allele. ΔAF is the change in allele frequency between fast and slow development rate C. macellaria. N is the average number of generated sequences for the sample group. Bold values are the variants and allele frequencies hypothesized to be associated with development rate.

				A				B			C		
Gene	Pos	Ref	Alt	Base Alt AF	Fast Alt AF	Slow Alt AF	Δ AF	Fast Alt AF	Slow Alt AF	Δ AF	Fast Alt AF	Slow Alt AF	Δ AF
Itgbn	1381	A	C	0.50	0.32	0.37	0.05	0.33	0.17	0.16	0.67[C]	0.13[C]	0.54
Itgbn	1437	A	C	0.49	0.51	0.18	0.33	0.57	0.50	0.07	0.10[C]	0.13[C]	0.03
Itgbn	1439	C	A	0.33	0.53	0.65	0.12	0.67	0.50	0.17	0.10[C]	0.13[C]	0.03
Itgbn	1442	A	G	0.49	0.50	0.64	0.14	0.29	0.58	-0.29	0.10[C]	0.25[C]	0.15
Smad2	1676	A	G	0.50	0.50	0.40	0.10	0.17	0.4	-0.23	0.45	0.70	0.25
Smad2	**1688**	**C**	**A**	**0**	**0.50**	**0**	**0.50**	0.66	0.5	0.16	0.45	0.10	0.35
Tsc1	3672	C	G	0.50	0.49	0.20	0.29	0.42	0.19	0.23	0.14	0.50[D]	-0.36
Tsc1	3846	C	T	0.50	0.50	0.35	0.15	0.79	0.31	0.48	0.79	0.75[D]	0.04
Tsc1	3951	T	C	0.50	0.67	0.35	0.32	0.14	0.57	0.43	0.79	0.50[D]	0.29
Ras	**956**	**A**	**T**	**0**	**0.50**	**0**	**0.50**	**0.80**	**0**	**0.80**	0	0	0
Ras	**980**	**G**	**A**	**0.35**	**0.65**	**0**	**0.65**	**0.67**	**0**	**0.67**	0.17	0.38	0.21
Ras	1037	G	A	0.50	0.46	0.47	0.01	0.08	0.64	0.56	0.33	0.93	0.60
Ras	1040	G	A	0.50	0.20	0.17	0.03	0	0.64	0.64	0.39	0.71	0.32
Ras	1043	C	T	0	0.52	0.17	0.35	0.92	0	0.92	0.17	0	0.17
Eip74EF	**467**	**G**	**A**	**0.50**	**0.77**	**0.17**	**0.60**	**0.92**	**0.36**	**0.56**	0.44	0.40	0.04
Eip74EF	488	G	A	0.50	0.28	0.27	0.01	0.34	0.29	0.05	0.61	0.50	0.11
rin	7738	T	G	0.33	0.51	0.39	0.12	0.38[A]	0.66[A]	0.28	0.25	0.50	0.25
EcR	**1066**	**A**	**T**	**0.64**	**1.00**	**0.20**	**0.80**	1.00	0.67[B]	0.33	0.88	0.95	0.07
Raptor	1230	A	G	0	0.70	0.23	0.47	0.57	0.71	0.14	0.44	0.57	0.13
Raptor	1236	G	T	0	1.00	1.00	0	0.14	1.00	0.86	1.00	1.00	0
Raptor	1240	A	C	0	1.00	1.00	0	1.00	1.00	0	1.00	1.00	0
Raptor	1244	C	G	0	1.00	1.00	0	1.00	1.00	0	1.00	1.00	0
InR	52	*	CGC	0.37	0.34	0	0.34	N/A	N/A	N/A	N/A	N/A	N/A
Babo	397	A	T	0.50	0.18	0.25	0.07	0.20	0	0.20	0.33	0.45	0.12
Babo	**505**	**G**	**A**	**0.50**	**0.50**	**0**	**0.50**	0.30	0.07	0.23	0.39	0.30	0.09
Babo	589	C	G	0.50	0.64	0.40	0.24	0.67	0.58	0.09	0.72	0.50	0.22
Babo	595	A	G	0.49	0.22	0.20	0.02	0.17	0.50	0.33	0.28	0.30	0.02
Babo	598	G	T	0.57	0	0.20	0.20	0.16	0.36	0.20	0.22	0.45	0.23
Babo	631	A	C	0.50	0.50	0.71	0.21	0.67	0.50	0.17	0.67	0.45	0.22

[A] *rin* generated 2 fast sequences and 2 slow sequences
[B] *EcR* generated 3 slow sequences
[C] *Itgbn* generated 6 fast sequences and 5 slow sequences
[D] *Tsc1* generated 7 fast sequences and 4 slow sequences

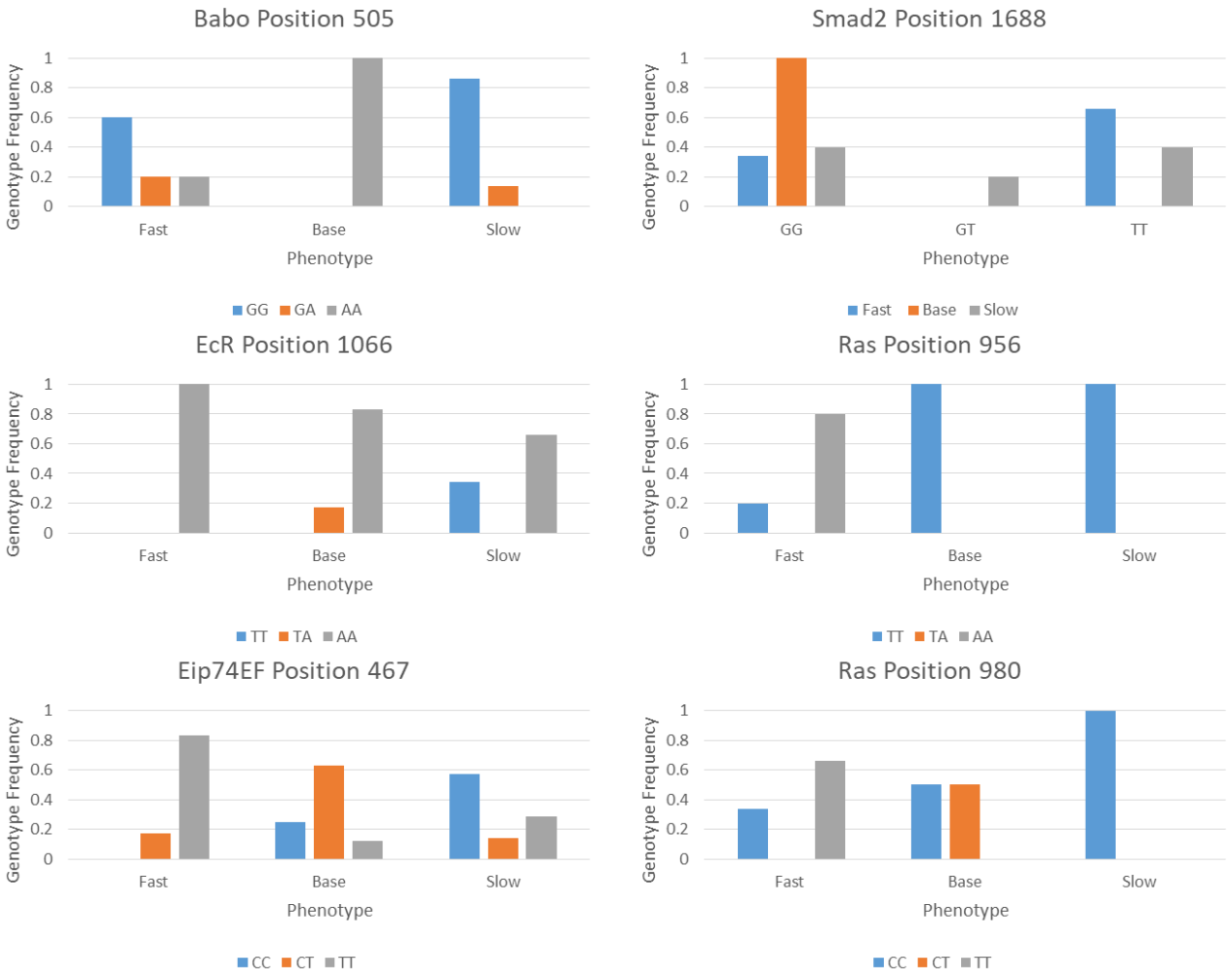

Figure 2. Phenotype and associated genotypes from the resequencing of baseline, fast developing, and slow developing *C. macellaria*. The six variant positions were theorized to be associated with development rate from the genome comparison.

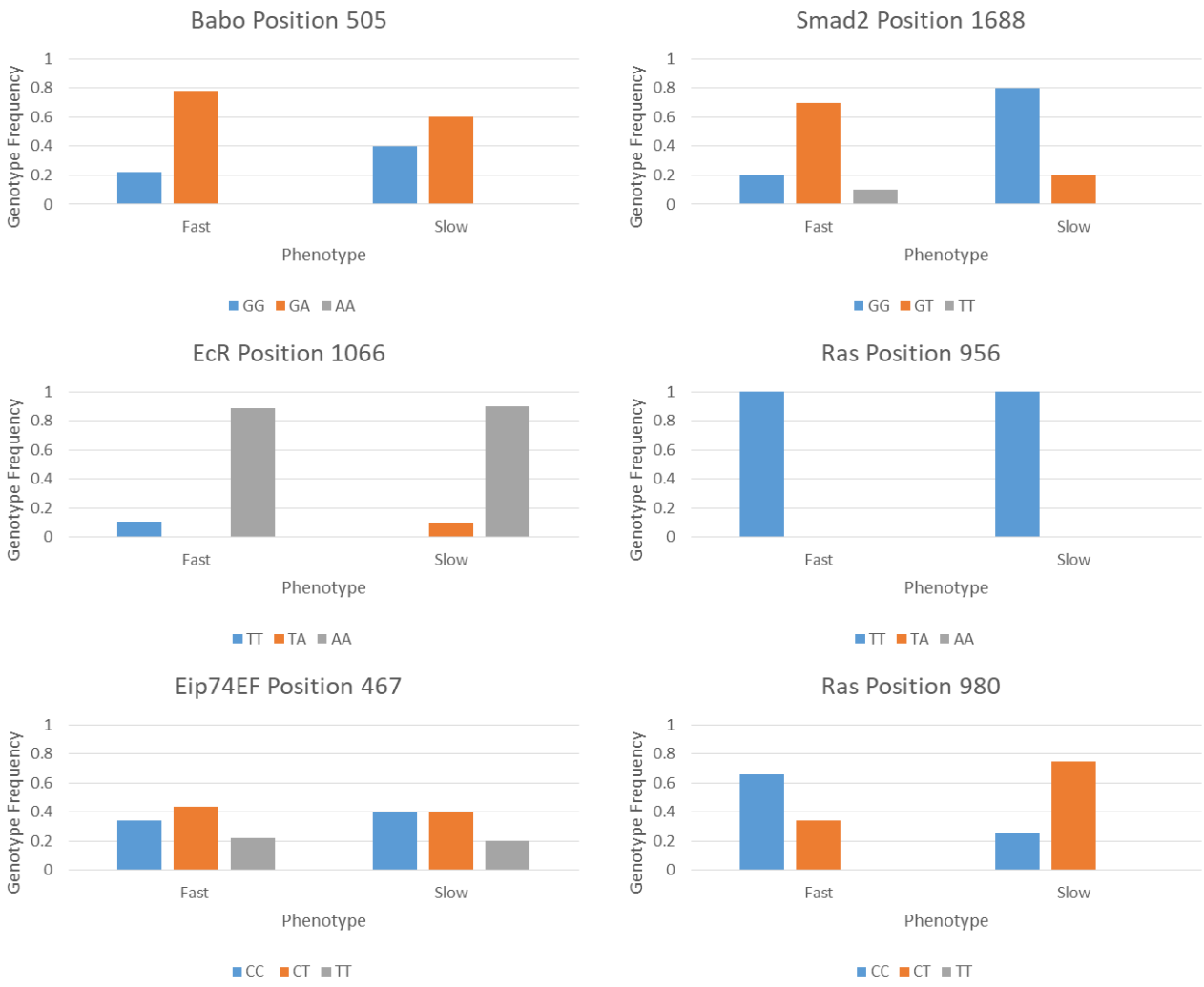

Figure 3. Phenotype and associated genotypes from the sequencing fast and slow developing offspring of wild *C. macellaria*. The six variant positions were theorized to be associated with development rate from the genome comparison.

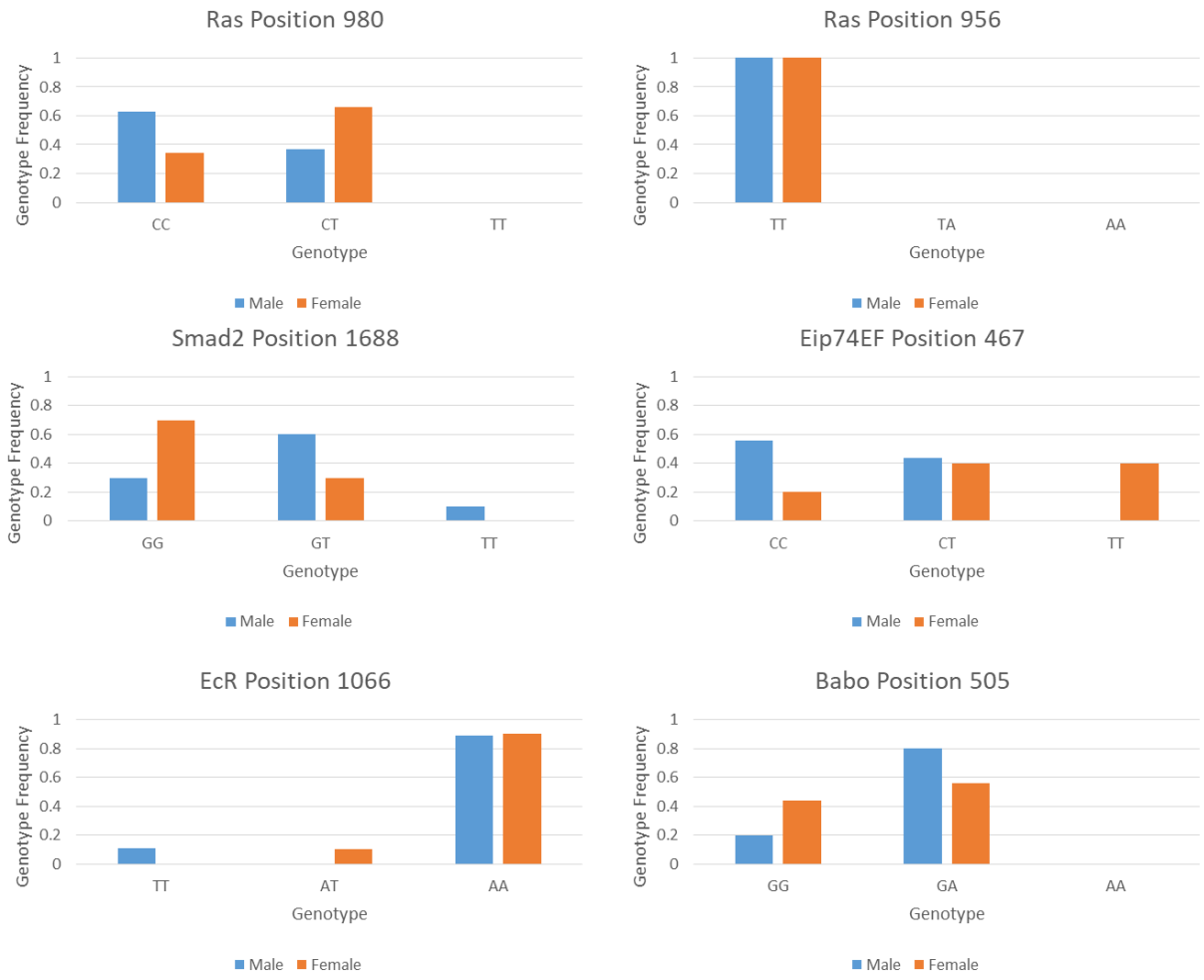

Figure 4. Phenotype and associated genotypes from the sequencing fast and slow developing offspring of wild *C. macellaria*. The six variant positions were theorized to be associated with development rate from the genome comparison.

2.4 Discussion

The investigation to identify variants associated with development rate started with known *D. melanogaster* developmental regulatory genes and their structure. Using gene ontology terms and research in development rate control, 57 genes were selected. The 57 genes were a part of the controlling factors for development rate and timing in *D. melanogaster*, and were used as a basis for investigating development rate variation in *C. macellaria*. From the 57 genes selected that were involved in development rate and timing, 10 genes contained variants in well conserved regions and were considered for downstream analysis. Five of the genes are believed to be involved in

controlling the production of ecdysone, two are involved in transcription and regulation events following ecdysone upregulation, and the final three are transcription factors and signaling mediators within other development pathways. Investigating genes known to be involved in development rate regulation provided a foundation to identify divergent variants that are associated with fast or slow development rate.

2.4.1 Enzymes in MAPK pathway effect development rate

The production of ecdysone controls the start and progression through metamorphosis (Yamanaka et al., 2013). To regulate the production of ecdysone, five pathways monitor the external and internal environment to time the start of metamorphosis for when the environmental conditions are right. Photoperiod is an environmental cue that stimulates the production of prothoracicotropic hormone (PTTH) in neurosecretory cells (McBrayer et al., 2007). This hormone starts a signaling cascade through the MAPK pathway to increase transcription of Halloween genes, such as the *shadow* which converts ecdysone from 2-deoxyecdysone, and initiates metamorphosis (Gilbert, 2008; Rewitz, Yamanaka, Gilbert, & O'Connor, 2009). PTTH activates membrane bound tyrosine kinase (torso) which then activates the downstream signaling protein GTPase raspberry (Ras). Reducing the expression of *Ras* in the MAPK pathway can delay pupation up to 4.3 days (Rewitz et al., 2009). *Ras* contained two synonymous variants from comparing the fast and slow genomes that potentially associated with development rate. Mutations in the *Ras* gene may have led to changes in the effectiveness of the MAPK signaling pathway which would influence transcription and upregulation of ecdysone production (Yamanaka et al., 2013).

2.4.2 Enzymes in activin pathway effect development rate

The production of ecdysone is regulated by the Activin signaling pathway in the prothoracic gland (PG). Activin signaling is stimulated by the binding of the activin ligand to the

membrane bound proteins Babo and Punt, intracellular signal-transducers in the activin pathway (Brummel et al., 1999). The two proteins form a type II complex with activin ligands to downregulate the activin pathway along with receptor regulated Smad2 (rSmad2) through phosphorylation (Peterson et al., 2012). Activated rSmad2 binds common partner SMAD (Medea) accumulating in the nucleus to increase transcription of cytochrome P450 Halloween genes that encode cytochrome P450 enzymes (Ying Y Gibbens et al., 2011). These enzymes are involved in ecdysteroid biosynthetic pathways with embryonic development and metamorphosis (Gilbert, 2004). The activin pathway also works to increase the sensitivity of the MAPK pathway and the insulin pathway in the PG cell. Inulin and Tor pathways require activin for normal expression to provide competence to PTTH and nutrient cues (Rewitz, Yamanaka, & O'Connor, 2013). Mutations within *Babo* and *Smad2* could result in delays of ecdysteroidogenesis, which would delay the onset of metamorphosis as observed with the development selection strains. Both of these genes contained synonymous variants that differentiated fast and slow strain *C. macellaria*.

2.4.3 Ecdysone-induced proteins effect development rate

After the hormone ecdysone is upregulated in PG cells, it is activated by an oxidation reaction and then binds to the EcR and USP proteins. USP and EcR form a nuclear ecdysone receptor which modulates the transcriptional activity of genes depending on the blow fly development stage and the larval tissue type the receptors are located (Davis et al., 2005). EcR actively promotes metamorphosis and development several times during the prepupation and pupation stages of development (T. Li & Bender, 2000). The EcR and USP heterodimer is responsible for transcriptional activation of ecdysone-induced proteins, such as the isoforms 74E and 74F encoded in *Eip74EF*. The main function of *Eip74EF* is the formation of puparium and autophagy. E74A and E74B work together to activate and inactivate different genes depending on

their levels during pupation (Fletcher, Burtis, Hogness, & Thummel, 1995). Mutations, other than the synonymous variants used in this study, within *EcR* could delay transcriptional activation of genes similar to *Eip74EF*. In this study EcR had the greatest change in allele frequency when comparing the genome out of all the variants tested, making it a strong locus for differentiating fast and slow developers. The change in allele frequency decreased greatly in the selection strain *C. macellaria* as well as the wild offspring. Stalling EcR production and activity would then delay the pupation process and elongating the development rate. Similar results may be seen when mutations occur in genes such as *Eip74EF*. The change in allele frequency was large between the fast and slow genomes, 0.6, as well as the fast and slow selection strains, 0.56. This trend did not continue for the wild offspring where the alternate allele frequency was only 0.04 greater for fast developers. Variants present in *Eip74EF* might lead to mutations in E74 and E75, affecting the activation rate of gene transcription.

2.4.4 Novel variants identified in sequencing

Only six variants from the original 29 identified when comparing the fast and slow genome were used to test the theory that variants were associated with development rate. Future research is needed to investigate the all of variants that effect development rate variation, not just the set of variants chosen for this study. Unidentified variants in the same 10 genes might have experienced a large change in allele frequency. One variant not a part of the original 29 variants was in the gene *rin*, at position 7758, in the selection strain sequences and had a change in allele frequency greater than 0.5. In the wild offspring the gene *Itgbn*, at position 1379, had a change in allele frequency greater than 0.5. The approach used in this study of selecting genes known to influence the phenotype in in question and then searching for genetic variants between the divergent strains is a faster and cheaper method than sequencing whole genomes of blow flies and comparing

individuals to isolate variants. There are drawbacks to this approach as well. The 10 genes selected are not the only genes that influence development rate variation. The variants investigated in this study are not strongly correlated to development rate of blow flies in the wild population as represented in Table 3. When investigating allele frequency changes in relation to sex, the frequency of the variants did not strongly correlate with male or female *C. macellaria* (Figure 4). This observation is supported when observing if sex had any influence on development rate. Males were observed to develop about five hrs faster than females, the impact on sex on development rate was considered insignificant (Ramos III, 2015). Studying genes known to be involved development regulation is only the beginning of understanding the underlying genetic factors that influence development rate variation.

2.5 Conclusion

From the initial 57 genes involved in development rate regulation, six variants were identified and theorized to be associated with the fast or slow developing phenotype. The variant alternate allele frequencies deviated between the fast and slow genomes and in the fast and slow development strains. A similar trend in alternate allele frequencies was observed in the wild offspring, but the difference in alternate allele frequencies were not as extreme in the wild offspring. It should be noted that these variants were all synonymous and not likely to be the cause of development rate variation. This is just a fraction of the genes known to be involved in development regulation and these variants could be used to establish a greater understanding of the genetic component of development variation. A different approach could be used to first identify significant variants that differ between selected phenotypes and then test to see if these variants produce similar results in wild offspring.

3. GENOMIC REGIONS EFFECTING DEVELOPMENT RATE

3.1 Introduction

Forensic entomologists focus research on the development of forensically relevant blow flies (Diptera: Calliphoridae) due to their role in providing information in minimum postmortem interval estimates (PMI_{MIN}). Developmental data generated in labs is used to help investigators estimate the age of the insects collected at crime scenes, and in turn, estimate the PMI_{MIN} (Byrd & Castner, 2010). The time it takes for the larvae to reach the different developmental stages varies, which can change the PMI_{MIN} and influence the investigation (Núñez–Vázquez, Tomberlin, Cantú–Sifuentes, & García–Martínez, 2013). Current studies focus on factors such as temperature changes, larvae density, blow fly species, and relative humidity and the influence on development time (Byrd & Butler, 1996, 1997; Johnson, 1975; Reigada & Godoy, 2006; A. M. Tarone et al., 2011). Published data indicates that blow flies of the same species raised under the same conditions differed in development time from published data, supporting the concept that there are developmental differences inter- and intra-species (Barros-Cordeiro, Pujol-Luz, & Báo, 2016). Other studies showed that offspring of the same species of blow fly collected from different geographic regions raised under the same abiotic conditions developed at different rates (Gallagher et al., 2010; C. G. Owings et al., 2014; A. M. Tarone & Foran, 2006). This supports the concept that genetics, along with environmental factors, contribute to development rate variation. The development of the blow fly is a well-known and well conserved process that is used by forensic entomologists to estimate the PMI_{MIN} (Byrd & Castner, 2010; Catts & Goff, 1992). The time it takes for blow flies to reach the different stages of their life cycle are recorded in lab based settings (Ames & Turner, 2003; Nabity et al., 2006). Blow flies used as forensic evidence are exposed to a variety of changing environmental factors which may differ from the laboratory study used to

determine their age (Arnaldos, Garcia, Romera, Presa, & Luna, 2005; Faris, Wang, Tarone, & Grant, 2016). Selecting the dataset the resembles the conditions that the entomological evidence experienced as well as including possible genetic contributions will lead to an increase in the precision of PMI_{MIN} estimates.

Majority of studies today are using next-generation sequencing techniques to observe the influence of genotypes on phenotypes throughout the genome. Whole genome sequencing involves the sequencing of individual's entire genome at one time. Analyzing sequencing data using this method is time consuming and expensive. A cheaper and complimentary approach to whole genome sequencing is the E&R (Burke et al., 2010; Nuzhdin et al., 2007). This approach typically pools extracted DNA from phenotypic strains after selection to form pooled genomes. In this study the E&R approach was used to observe genetic variation that correlated to changes in development rate. E&R studies can be used to locate genomic changes associated with the selected phenotype and then were able to use gene ontology to identify what parts of the genome were responsible for the variation. The E&R approach has been used by *D. melanogaster* researchers for observing genetic links to selected phenotypes such as courtship song, body size, egg size, and accelerated development (Chippindale, Alipaz, Chen, & Rose, 1997; Jha et al., 2015; T. L. Turner & Miller, 2012; Thomas L. Turner et al., 2011). These studies located genomic changes as a result of artificial selection. When studying the genetic variations when selecting for body size, the regions and genes associated with the phenotype were identified and characterized (Thomas L. Turner et al., 2011). When observing the changes in the genome after selecting for courtship song, the identified variants in the pooled genomes were was little spatial clustering of the variants (T. L. Turner & Miller, 2012).

The objective of this study was to investigate what parts of the genome are responsible for development rate variation in *C. macellaria*. Pooled sequencing of the fast and slow developing strains (two geographic replicates) from the artificially evolved selection experiment (unpublished) were generated. The genomic data from the pooled genomes were used to identify the biological process and molecular functions involved in development rate variation. This study should provide insight into which genes are involved in development rate variation.

3.2 Materials and Methods

3.2.1 Description of genomes used

Trimmed paired Illumina reads from a previous selection experiment were used to create four genomes (Ramos III, 2015). The *C. macellaria* ancestral populations used for the selection experiment consisted of two geographic replicates, College Station, Texas and Longview, Texas. Each geographic replicate experienced the same artificial selection pressures creating a "slow" developing selection strain and "fast" developing selection strain. The four genomes were labeled College Station Slow (CSSlow), College Station Fast (CSFast), Longview Slow (LVSlow), and Longview Fast (LVFast). The reads were mapped to a reference draft genome generating 375,920 contigs (543 Mbp) created from the pooled slow reads using both geographic replicates with Burrows-Wheeler Aligner (BWA) v0.7.12 mem for maximum exact matches and extending seeds using affine-gap Smith-Waterman (H. Li & Durbin, 2010). This algorithm compares the sequence segments and of varying lengths and optimizes the similarity measure, quantifying the similarity of the sequence segments.

3.2.2 Variant discovery

GNU Complier Collection (GCC) v6.3.0 was used to support the operation of genome analysis tools, such as SequenceAlignment/Map tools (SAMtools) v1.5. SAMtools is set of utilities that can be used to manipulate genomes in the SAM format, such as indexing alignments, sorting, and alignment information per position (H. Li, 2011; H. Li et al., 2009). The alignment tool produced SAM files which were then converted into BAM files and sorted by the contig name. The BAM files were indexed by coordinate name to allow for regions arguments in future analysis. The BAM files were converted into a single mpileup file. The mpileup file produced information for each position in the BAM files, including information such as variants, mapping quality, and frequency of variant. The java script from Popoolation2 was used to sort the combined mpileup file so each contig and position had the information of both geographic replicates and selection strains.

3.2.3 Genome wide association study

A Cochran-Mantel-Haenszel test (CMH test) was run to detect consistent allele frequency changes in the geographic replicates. This method was used for the significant test because it takes into account the independent measures of allele frequencies obtained, such as using geographic replicates. The CMH test reports significance values only for consistent allele frequency changes at shared loci between the fast and slow developing strains. Q-value estimation for false discovery rate (FDR) v2.10.0 (Bioconductor) package in RStudio was used to estimate the *q*-values given from the CMH test significance values for each variant. The FDR cutoff value for significant loci that deviated between the fast and slow genomes was 0.001, indicating that 0.1% of the variants that were called significant are false positives. A list of contig names that contained the significant

loci was generated. A file of the sequences identified by the contig names was created using a perl code.

3.2.4 Functional characterization

Annotation and gene oncology analysis was performed using Blast2GO v5.0.21 (Conesa et al., 2005). NCBI blast (QBlast) was chosen for its access to public databases. The blast program chosen was the discontinuous megablast (dc-blastn). The database the sequences were searched against was the non-redundant database with an Arthropoda taxonomy filter. The blast expectation value was set for 1.0E-5 to exclude less stringent matches to the sequences being blasted. Results with BLAST hits were searched against the gene ontology (GO) annotated protein database. The sequences were annotated for the GO terms related to the blast results. The annotation was run with a filter at 1E-10 to produce more conservative annotation results using Blast2GO. GO graphs were created using Blast2GO to demonstrate annotation results and the biological processes, molecular functions, and cellular components effected by the selection for development rate. Enrichment Analysis using Fisher's Exact Test in Blast2GO was run using a FDR of 0.001 and the 219 sequences that resulted in annotation results were listed as the test-set files. The enrichment results were reduced by applying another FDR of 0.001 to remove parent GO terms of statistically significant child GO terms. The Kyoto Encyclopedia of Genes and Genomes (KEGG) used enzyme codes from the Blast2GO to identify where the sequences fit into enzymatic pathways. Reference sequences for the genome region of RNA polymerase II, elongation factor, Cyp12A2, and the four AS-C genes were collected from Flybase (Gramates et al., 2017). *Cochliomyia macellaria* sequences connected to these genes were aligned to the reference sequences using Clustal Omega to identify aligned sequences and location of significant variants, and if these variants were associated with the Flybase reference sequences (Sievers et al., 2011). The *C.*

macellaria sequences from all four genomes with the greatest number of variants in the biological process, molecular function, and blast results from Blast2GO, had their structure predicted using Augustus (Stanke et al., 2004).

3.3 Results

3.3.1 Selection strain genome comparison

The contig, position, and the allele frequencies (A-count: T-count: C-count: G-count: N-count: deletion-count) for each of the four genomes were matched so the allele frequency could be compared between replicates. Using CMH the significance the change in allele frequency for each position was calculated into a *P* value for a total of 645,822 variants in 18,179 contigs shared between the replicates that differentiate development rate, not geographic differences. Converted significance values were treated with a FDR of 0.1% yielding 7,290 variants in a total of 699 contigs. When observing the base pairs in the reference draft genome, 0.00134% were significant variants. A list of the 699 contig names was created from the CMH test and the sequences in the reference draft genome were compiled into a separate file.

3.3.2 Functional characterization of sequences containing significant variants

From the 699 sequences with significant variants, Blast2GO analysis resulted in 11 sequences that did not run (1.57%), 156 sequences without blast hits (22.32%), 231 sequences with nucleotide blast hits (33.05%), 82 sequences that had nucleotide blast hits and protein blast hits (11.73%), and 219 sequences that had gene ontology annotations (31.33%). Sequences that did not result in blast analysis results were either too short in length, or produced E values above the default threshold. The top four species that reported similar sequences in blast results came from *Lucilia cuprina* Meigen (Diptera: Calliphoridae) (1,677 hits), *Calliphora vicina* (657 hits),

and *Drosophila virilis* Sturtevant (Diptera: Drosophilidae) (380 hits), and *D. melanogaster* (364 hits) out of 6,894 blast hits in 220 arthropod species (Figure 5). A portion of the blast results, 69 out of 219, were *Achaete-Scute* clone BAC, a group of four genes that when transcribed form helix-loop-helix transcription factors for the development of the nervous system (García-Bellido & de Celis, 2009). Alignment of the *C. macellaria* sequences did not associate any of the 69 sequences with the four genes. The second most numerous blast result from the annotated sequences was *Cyp12A7*, a cytochrome P450 gene, with 14 out of 219 sequences yielding blast hits. None of the 14 sequences aligned to the reference *Cyp12A2* sequence.

3.3.2.1 Biological processes, molecular functions, and enzyme pathways effected

The most represented cluster of biological processes (GO terms) included genes effecting developmental processes, multicellular organismal processes, multicellular organismal development, and anatomical structure development (Figure 6). The molecular function analysis results reported transporter activity with branches in organic transmembrane transporter activity, inorganic molecular entity transmembrane transporter activity, and ion/anion transmembrane transporter activity (Figure 7). When applying Enrichment Analysis using Fisher's Exact Test and reducing results, the number of biological processes decreased from 1,045 hits to 177 hits with associated *P* values (FDR $\leq$ 0.0001). The most significant biological process was regulation of transcription by RNA polymerase II ($P < 0.0001$). The alignment of sequences associated with regulation of RNA polymerase II activity did not align with RNA polymerase II subunits or elongation factor. Molecular function hits decreased from the original count of 280 hits to 109 hits with associated *P* values (FDR $\leq$ 0.0001), the most significant result being protein dimerization activity ($P < 0.0001$). A total of 116 KEGG maps from the Blast2GO analysis were identified. Figure 8 is a visual representation of the 29 molecular pathways that contain 10 or more *C.*

macellaria sequences. Of the 29 molecular pathways in Figure 8, 17 of the pathways are involved in metabolism. Two of the pathways are purine metabolism and pyrimidine metabolism, that pathways that produce the ring structures for nucleotides.

Investigating the structure of sequences containing the greatest number of variants across the four genomes involved 16 contigs, four sequences from each selection strain genome for the biological process, molecular function, and blast results. The sequence with the greatest number of variants in the biological process of regulating transcription of RNA polymerase II was 21240 with five variants. The significant molecular function was protein dimerization activity, and the *C. macellaria* sequence related to this function was 14924, containing nine variants. The two most numerous blast results were AS-C with the sequence 10538 containing six variants, and Cyp12A7 with the sequence 7518 containing four variants. No structure was predicted for the four sequences containing the greatest number of variants in all four genomes.

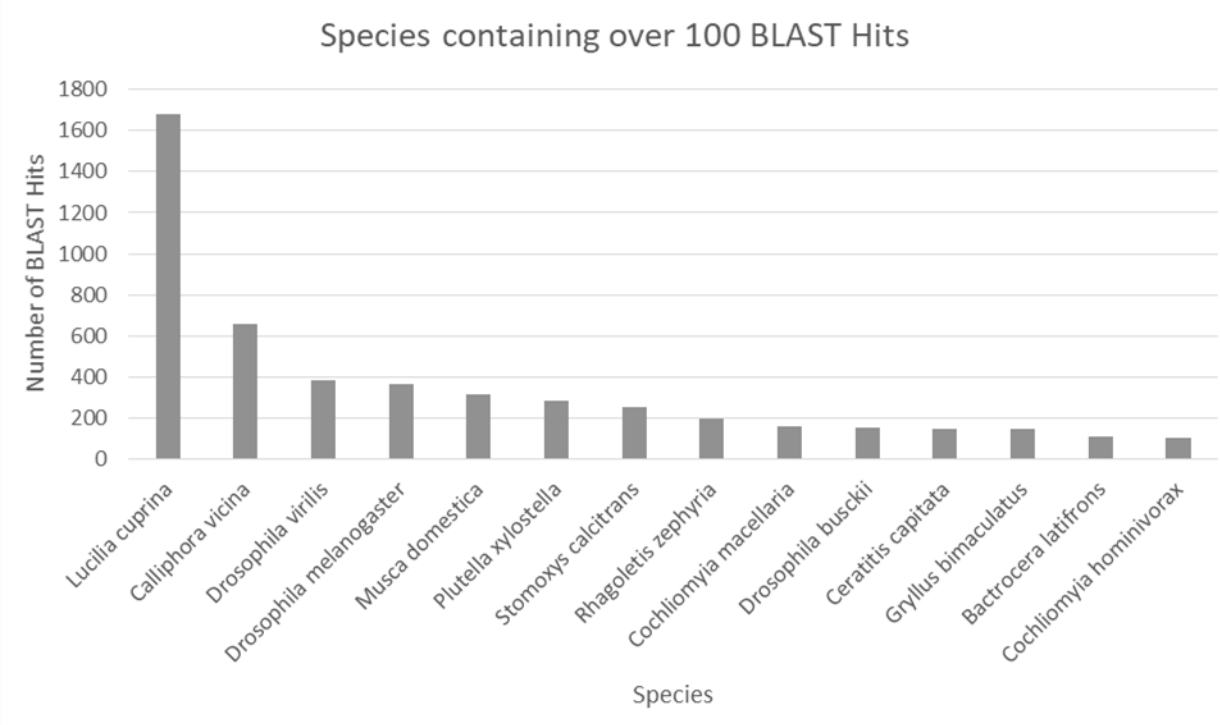

Figure 5. The top 14 species in BLAST results containing more than 100 hits.

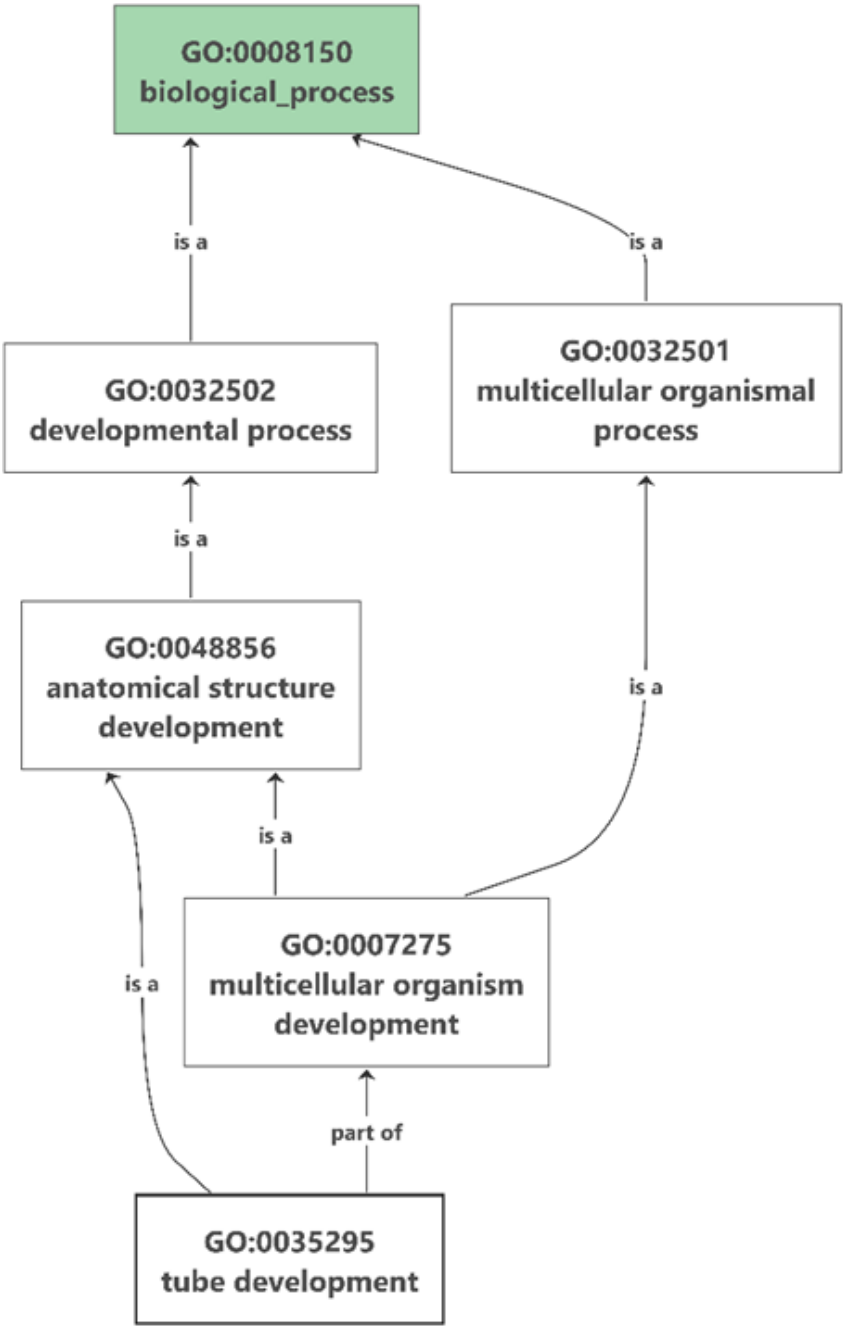

Figure 6. Biological process ontology results for the 219 sequences with annotation results. The two processes represented include developmental process for an organism over time and multicellular processes that are pertinent to multicellular organismal function

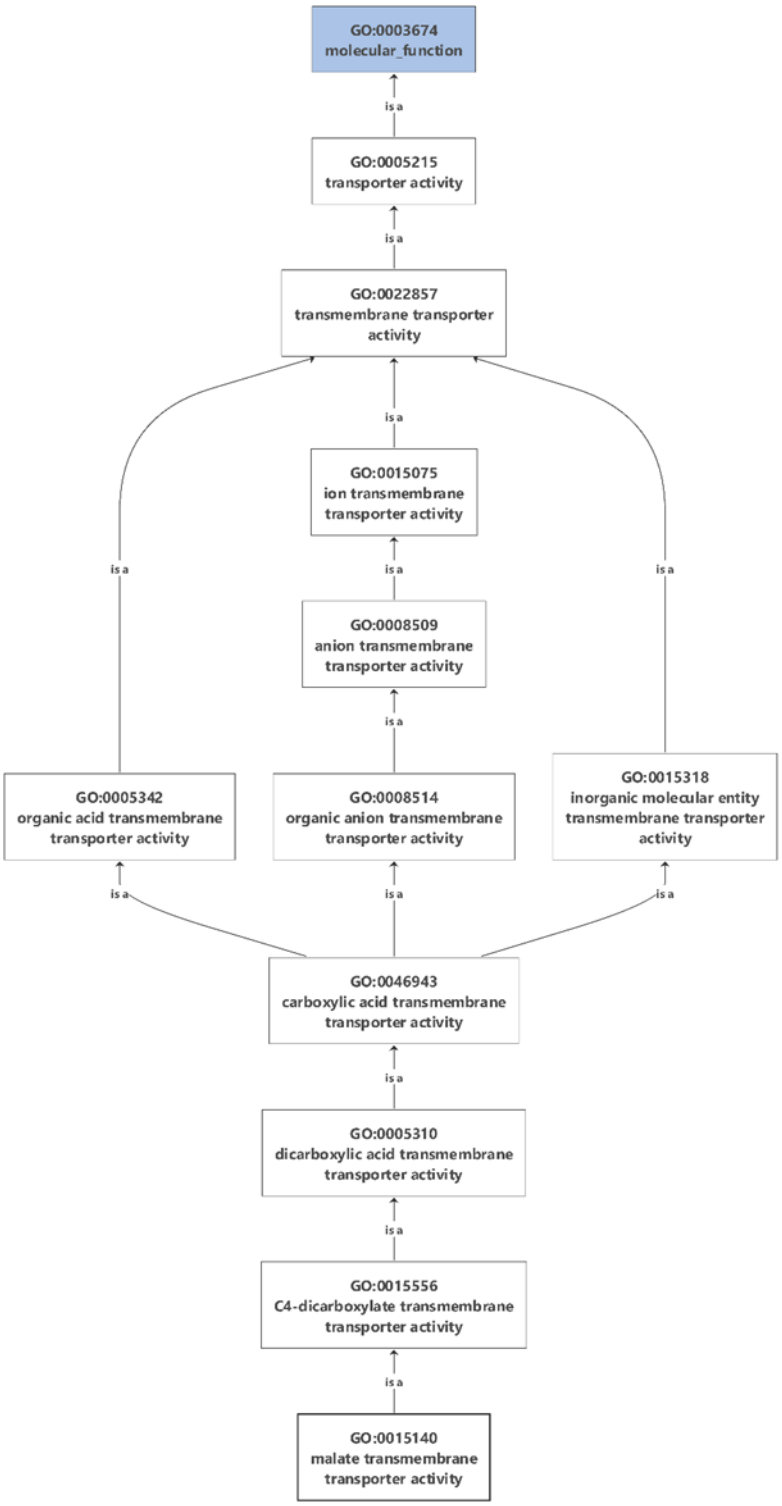

Figure 7. Simplified molecular functions that categorized annotated sequences. The overall molecular function for the sequences is transmembrane transporter activity.

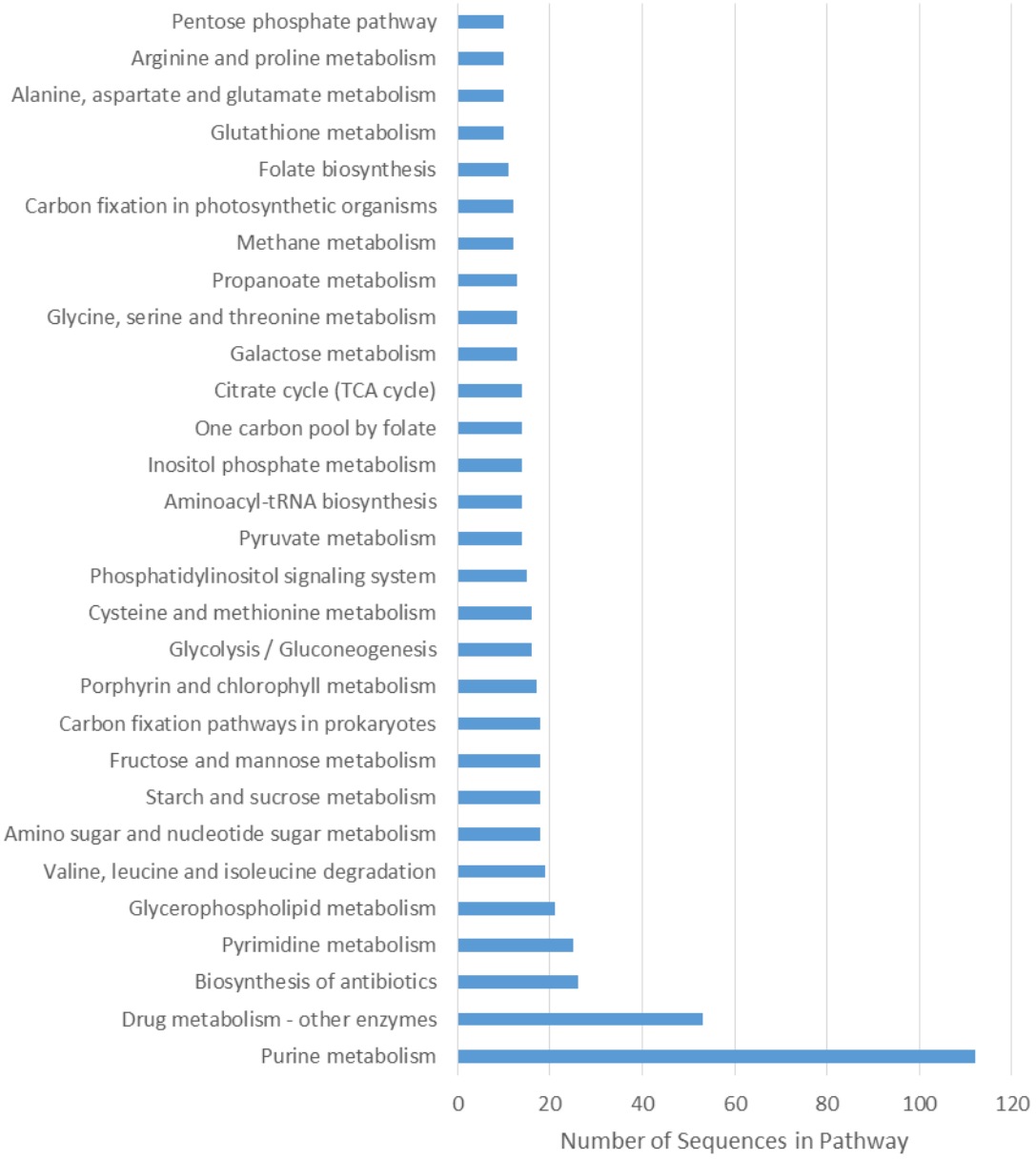

Figure 8. The 29 KEGG biological pathways of the sequences differentiated between the fast and slow genome extracted from Blast2GO analysis.

3.4 Discussion

Some considerations must be taken into account when analyzing results from an E&R study to isolate genomic variants between artificially selected strains. Variants in between the selection strains indicate that the phenotype examined, development rate, is polymorphic. A study that applied the same approach observed the phenotype body size. The body size variation E&R study had 5,205 peak variants in multiple coding regions, and these results support the theory that the phenotype development rate will also produce variants in multiple regions (Thomas L. Turner et al., 2011). Pleiotropic effects of should be taken into consideration when making observations of variants and their associated GO term. An example of pleiotropic effect when selecting for faster development rate is the production of smaller flies in a response to shortening the time taken in each instar (Caldwell et al., 2005). A study found that when comparing the control *D. melanogaster* to the faster developing *D. melanogaster*, the larval development time was reduced by 7.9%. The trade-off for developing faster was a decrease in in adult mass by 15.1% when compared to adult controls (Nunney, 1996). This trade-off between size and development rate occurred during the selection experiment, causing variants related to smaller body size to increase in frequency along with variants related to faster development (Ramos III, 2015).

3.4.1 Regulation of RNA polymerase II

The sequences containing variants related to development rate variation between fast and slow strain genome were involved in biological processes, mainly development process, as shown in Figure 6. When observing the significant GO terms in biological processes from the enrichment analysis, the GO term with the highest significance was regulation of transcription by RNA polymerase II. RNA polymerase II regulated DNA transcription by binding to specific DNA sequences to selectively activate transcription of a specific gene (Kadonaga, 2004). Gene

transcription starts with the binding transcription factors at upstream gene promotors before RNA polymerase II attaches to the DNA and elongation of the mRNA can begin. The sequences that partially aligned to each other were poor quality with large amounts of indels and SNPs. A total of 119 variants were located within the 97 sequences related to RNA polymerase II without clustering in one particular sequence. Position 46 in sequence 21240 had a change in allele frequency of A greater than 0.5 in both geographic replicates. This locus would be applicable for predicting if a blow fly was fast or slow developing, with adenine associated with slow development and thymine associated with fast development. Sequences related to RNA polymerase II were aligned with *D. melanogaster* RNA polymerase II subunits and elongation factor sequences, and no alignment was observed indicating that the variants are not located in the RNA polymerase II enzyme or the elongation factor. The variants may be located within a network of thousands of sequence-specific DNA binding transcription factors which interpret the DNA regulatory information such as enhancing or promoting sequences, and relay this information to RNA polymerase II (Kadonaga, 2004). Mutations within these regulatory sequences could influence the capability of the transcription factors to communicate to RNA polymerase II. One pathway that increases RNA polymerase II activity is the Mitogen-activated protein kinases (MAPK) pathway. The MAPK pathway connects cell surface receptors to regulatory components of the cell. The MAP kinases bind directly to target genes which may or may not recruit positive transcription factors to encourage transcription of target genes related to ecdysone production (Pokholok, Zeitlinger, Hannett, Reynolds, & Young, 2006).

3.4.2 Protein dimerization

The annotated molecular function results of the artificial selection for development rate correlated to sequences that transcribed transporters, with a focus on transmembrane transporters

as shown in Figure 7. The insulin signaling pathway uses transmembrane transporters, such as insulin-like receptor (InR), so the extracellular signal insulin can influence the inside of the PG cell. The enriched molecular function GO term with the highest probability of being significant was protein dimerization activity. A protein dimer is formed when to two proteins associate together to form a complex. An example of a transmembrane protein dimer receptor would be insulin receptor (InR) which is composed of 2 alpha-beta monomers (Van Obberghen et al., 1981). InR transports the insulin signal into the prothoracic gland (PG) cells for the monitoring the nutrient status of the blow fly larvae. When the nutrient barrier is reached the larvae will proceed through metamorphosis by the upregulation of ecdysone. Mutations that occur in transmembrane dimer proteins like InR could alter the effectiveness of the insulin signal reaching the inside of the PG cell, leading to delays in metamorphosis. One study showed that mutations in the *InR* gene could cause severe developmental delays for up to 10 days and a growth deficiency with a lower rate of cell proliferation. *Drosophila melanogaster* with mutant InR proteins eclosed eight to nine days at 25 °C after the flies containing a copy of the wild type *InR* gene (Chen, Jack, & Garofalo, 1996). This supports the theory that variants in coding sequences for transmembrane signaling proteins, including protein dimers, can effect development rate.

3.4.3 Neural development transcription factors

The results from the blast analysis on the selection strain sequences indicated similar structured arthropod genes that may have an effect on the development rate of the blow fly. Regulating DNA transcription by influencing binding sites of transcription factors for RNA polymerase II may decrease the rate proteins involved in development are being transcribed. Achaete-Scute, 31.96% of the gene blast results, contains four proneural genes (*achaete, scute, lethal of scute,* and *asense*) that transcribe as Achaete-scute transcription factors. These four

transcription factors commit epidermal cell toward a neural fate, such as sensory organ development and neuroblasts in embryos of *D. melanogaster* (García-Bellido & de Celis, 2009). The Achaete-Scute complex (AS-C) is required for neural development of the central and peripheral nervous system, which is a part of anatomical structure development in Figure 6. The enhancer sequences around the AS-C are conserved across *D. melanogaster* and *C. vicina* that bind known transcription factors (Negre & Simpson, 2015). Located within the 69 sequences are 95 variants, and none of the 69 sequences aligned to *achaete, scute, lethal of scute*, and *asense*. The alignment results suggests that the effect of artificially selecting for divergent development rates was minor, and did not influence one transcription factor gene over the other four. Mutations within binding sites of the AS-C would affect neural development and downstream processing of cellular patterning, but minor changes as seen in the variants changes may not yield lethal phenotypes. Delays in neural development could lead to delays in overall development, and increasing development variation.

3.4.4 Cytochrome P450 genes

Blast results indicated that *Cyp12A7*, a part of the cytochrome P450 family, may be related to development rate variation. The 14 sequences that share similarities to *Cyp12A7* contained 21 variants. Cytochrome P450 genes are a family of genes that are involved in synthesis and breakdown of various molecules in the cell. The family of genes also synthesize and breakdown fatty acids and digestive acids (Hannemann, Bichet, Ewen, & Bernhardt, 2007). Common variants with the cytochrome P450 genes can affect the functionality of the enzymes in a variety of metabolic pathways in different regions of the cell. The function of the 12A subfamily was inferred from the similarities with *D. melanogaster* to code for mitochondrial P450s which transfer electrons in the oxidative-reduction process in mitochondria (Tijet, Helvig, & Feyereisen, 2001).

Variants in the sequences related cytochrome P450 differentiated between fast and slow developing *C. macellaria*, supporting the theory that variants effected the mitochondria are involved in development rate variation.

3.4.5 Metabolic pathways

Enzyme variants in metabolic pathways are likely to affect the development rate of a blow fly by influencing growth rate. Delays in purine and pyrimidine metabolic pathways can lead to delays in development. One study found that de novo purine synthesis limits growth rate, and the biosynthesis of purines increases growth rate through ATP production and transitioning the cell from G1 to S phase (Kondo et al., 2000). When pyrimidine synthesis is reduced, the growth rate of the organism is reduced (Schröder, Giermann, & Zrenner, 2005). A majority of the pathways that contained sequences that were differentiated between fast and slow *C. macellaria* involved the metabolism tie into processing the carrion to convert into energy and fat stores. These pathways may affect the digestion and breakdown of the resource, making it harder for the larvae to reach critical weight. With delaying the time taken to reach critical weight or de novo synthesis of nucleotide rings, variation in development rate would be observed.

3.5 Conclusion

The set of significant variants that differentiate between the fast and slow developing *C. macellaria* should be considered for further study in their frequency in wild populations. The sequences that contain the variants were investigated to discover which biological processes and molecular functions are being effected to cause the change in development rate. One locus was identified that had allele frequencies in both geographic replicates that were associated with fast and slow development. Further analysis should be done to characterize the variants identified.

Understanding if the variants are nonsynonymous and what the resulting change to the protein may increase the understanding in the molecular basis of development rate variation. Testing the validity of these variants using wild *C. macellaria* could provide a set of variants that can be used to classify a blow fly as fast or slow developing. This may help forensic entomologist decrease the range of their PMI_{MIN} estimates.

4. CONCLUSION AND FUTURE STUDIES

This study investigated the genetic components involved with development rate variation in the blow fly *C. macellaria* using two different approaches. The first approach was to locate variants in known genes associated with development rate regulation. Three variants identified from the fast and slow genome comparison and selection strain sequencing were associated with development rate. The wild offspring did not have a strong change in allele frequency at these variants making the application of these genetic markers difficult in forensic entomology casework. Due to the lack of difference in wildtype genomes, this study outlines a need for further investigation into allele frequencies in wild blow fly populations within specific genes associated with the phenotype being investigated. The second approach to investigating the genomic contribution to development rate variations in *C. macellaria* was to compare pooled geographic replicate genomes for fast and slow artificially selected blow flies. A majority of the variants were associated with regulation of RNA polymerase II transcription and protein dimerization, indicating that altering the genotype of these biological functions affect development variation. Specific genes that are involved in development rate variation include AS-C and *Cyp12A7*, providing evidence that altering the neural development and mitochondrial oxidation reduction pathways results in a change in development rate.

Further investigation into the annotation results from comparing the fast and slow developing genomes may increase the understanding of the molecular effects of the identified variants. The next step would be to investigate the protein structure changes resulting from nucleotide variants. Changes in structure could affect signal binding, protein dimerization, and transcription of development regulatory genes. Understanding which parts of the genome influence

development rate in *C. macellaria* will also increase the understanding of development rate variation in other blow fly species. With the identification of the genomic regions that influence development rate, genetic markers associated with the fast or slow phenotype can be identified and applied to PMI_{MIN} estimates. The use of two approaches to investigate development rate variation provided groundwork for future studies to build upon. Further analysis of gene related to development rate will increase the likelihood of locating genetic markers linked to fast or slow development.

REFERENCES

Ames, C., & Turner, B. (2003). Low temperature episodes in development of blowflies: implications for postmortem interval estimation. *Med Vet Entomol, 17*(2), 178-186.

Andrewartha, H. G., & Birch, L. C. (1986). *The Ecological Web: More on the Distribution and Abundance of Animals*: University of Chicago Press.

Arnaldos, M. I., Garcia, M. D., Romera, E., Presa, J. J., & Luna, A. (2005). Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Sci Int, 149*(1), 57-65. doi:10.1016/j.forsciint.2004.04.087

Ash, N., & Greenberg, B. (1975). Developmental Temperature Responses of the Sibling Species Phaenicia sericata and Phaenicia pallescens1. *Annals of the Entomological Society of America, 68*(2), 197-200. doi:10.1093/aesa/68.2.197

Bansode, S., More, V., & Zambare, S. (2016). Effect of different constant temperature on the life cycle of a fly of forensic importance Lucilia cuprina. *Entomol. Ornithol. Herpetol, 5*(3), 2161-0983.

Barros-Cordeiro, K. B., Pujol-Luz, J. R., & Báo, S. N. (2016). Intra-puparial development of the Cochliomyia macellaria and Lucilia cuprina (Diptera, Calliphoridae). *Revista Brasileira de Entomologia, 60*(4), 334-340.

Bialecki, M., Shilton, A., Fichtenberg, C., Segraves, W. A., & Thummel, C. S. (2002). Loss of the ecdysteroid-inducible E75A orphan nuclear receptor uncouples molting from metamorphosis in Drosophila. *Developmental Cell, 3*(2), 209-220.

Boatright, S. A., & Tomberlin, J. K. (2010). Effects of temperature and tissue type on the development of Cochliomyia macellaria (Diptera: Calliphoridae). *J Med Entomol, 47*(5), 917-923.

Brummel, T., Abdollah, S., Haerry, T. E., Shimell, M. J., Merriam, J., Raftery, L., . . . O'Connor, M. B. (1999). The Drosophila activin receptor baboon signals through dSmad2 and controls cell proliferation but not patterning during larval development. *Genes Dev, 13*(1), 98-111.

Brundage, A., Benbow, M. E., & Tomberlin, J. K. (2014). Priority effects on the life-history traits of two carrion blow fly (Diptera, Calliphoridae) species. *Ecological entomology, 39*(5), 539-547.

Burke, M. K., Dunham, J. P., Shahrestani, P., Thornton, K. R., Rose, M. R., & Long, A. D. (2010). Genome-wide analysis of a long-term evolution experiment with Drosophila. *Nature, 467*(7315), 587-590. doi:10.1038/nature09352

Byrd, J. H., & Butler, J. F. (1996). Effects of temperature on Cochliomyia macellaria (Diptera: Calliphoridae) development. *J Med Entomol, 33*. doi:10.1093/jmedent/33.6.901

Byrd, J. H., & Butler, J. F. (1997). Effects of temperature on Chrysomya rufifacies (Diptera: Calliphoridae) development. *J Med Entomol, 34*. doi:10.1093/jmedent/34.3.353

Byrd, J. H., & Castner, J. L. (2010). *Forensic entomology: the utility of arthropods in legal investigations*. Boca Raton: CRC Press.

Cáceres, L., Necakov, A. S., Schwartz, C., Kimber, S., Roberts, I. J., & Krause, H. M. (2011). Nitric oxide coordinates metabolism, growth, and development via the nuclear receptor E75. *Genes & development, 25*(14), 1476-1485.

Caldwell, P. E., Walkiewicz, M., & Stern, M. (2005). Ras Activity in the Drosophila Prothoracic Gland Regulates Body Size and Developmental Rate via Ecdysone Release. *Current Biology, 15*(20), 1785-1795. doi:https://doi.org/10.1016/j.cub.2005.09.011

Callier, V., & Nijhout, H. F. (2011). Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. *Proceedings of the National Academy of Sciences, 108*(35), 14664-14669.

Cassidy, J. J., Jha, A. R., Posadas, D. M., Giri, R., Venken, K. J., Ji, J., . . . Carthew, R. W. (2013). miR-9a minimizes the phenotypic impact of genomic diversity by buffering a transcription factor. *Cell, 155*(7), 1556-1567. doi:10.1016/j.cell.2013.10.057

Catts, E. P. (1990). *Analyzing entomological data*. Clemson, SC: Joyce's Print Shop.

Catts, E. P., & Goff, M. L. (1992). Forensic entomology in criminal investigations. *Annu Rev Entomol, 37*, 253-272. doi:10.1146/annurev.en.37.010192.001345

Chapman, R. F. (2012). *The insects : structure and function*: Cambridge University Press.

Chen, C., Jack, J., & Garofalo, R. S. (1996). The Drosophila insulin receptor is required for normal growth. *Endocrinology, 137*(3), 846-856.

Chippindale, A. K., Alipaz, J. A., Chen, H. W., & Rose, M. R. (1997). Experimental evolution of accelerated development in Drosophila. 1. Developmental speed and larval survival. *Evolution, 51*(5), 1536-1551.

Cianci, T., & Sheldon, J. (1990). Endothermic generation by blow fly larvae Phormia regina developing in pig carcasses. *Bulletin of the Society for Vector Ecology, 15*(1), 33-40.

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics, 21*(18), 3674-3676.

Cook, D. F., Voss, S. C., & Dadour, I. R. (2012). The laying of live larvae by the blowfly Calliphora varifrons (Diptera: Calliphoridae). *Forensic science international, 223*(1-3), 44-46.

Cyr, T. L. (1993). *Forensic implications of biological differences among geographic races of Phormia regina (Meigen)(Diptera: Calliphoridae).* Washington State University.

Davis, M. B., Carney, G. E., Robertson, A. E., & Bender, M. (2005). Phenotypic analysis of EcR-A mutants suggests that EcR isoforms have unique functions during Drosophila development. *Dev Biol, 282*(2), 385-396. doi:10.1016/j.ydbio.2005.03.019

de Carvalho, L. M. L., Linhares, A. X., & Palhares, F. A. B. (2012). The effect of cocaine on the development rate of immatures and adults of Chrysomya albiceps and Chrysomya putoria (Diptera: Calliphoridae) and its importance to postmortem interval estimate. *Forensic science international*, *220*(1-3), 27-32.

Dittmer, C. (1931). Animal Aggregations: A Study in General Sociology: JSTOR.

Dubrovsky, E. B., Dubrovskaya, V. A., & Berger, E. M. (2004). Hormonal regulation and functional role of Drosophila E75A orphan nuclear receptor in the juvenile hormone signaling pathway. *Dev Biol, 268*(2), 258-270. doi:10.1016/j.ydbio.2004.01.009

e Castro, C. P., Sousa, J. P., Arnaldos, M. I., Gaspar, J., & García, M. D. (2011). *Blowflies (Diptera: Calliphoridae) activity in sun exposed and shaded carrion in Portugal.* Paper presented at the Annales de la société entomologique de France.

Faria, L. D. B., Orsi, L., Trinca, L. A., & Godoy, W. A. C. (1999). Larval predation by Chrysomya albiceps on Cochliomyia macellaria, Chrysomya megacephala and Chrysomya putoria. *Entomologia Experimentalis et Applicata, 90*(2), 149-155. doi:10.1046/j.1570-7458.1999.00433.x

Faris, A. M., Wang, H. H., Tarone, A. M., & Grant, W. E. (2016). Forensic Entomology: Evaluating Uncertainty Associated With Postmortem Interval (PMI) Estimates With Ecological Models. *J Med Entomol*. doi:10.1093/jme/tjw070

Fletcher, J. C., Burtis, K. C., Hogness, D. S., & Thummel, C. S. (1995). The Drosophila E74 gene is required for metamorphosis and plays a role in the polytene chromosome puffing response to ecdysone. *Development, 121*(5), 1455-1465.

Foster, K. G., Acosta-Jaquez, H. A., Romeo, Y., Ekim, B., Soliman, G. A., Carriere, A., . . . Fingar, D. C. (2010). Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation. *Journal of Biological Chemistry, 285*(1), 80-94.

Fouche, Q., Hedouin, V., & Charabidze, D. (2018). Communication in necrophagous Diptera larvae: interspecific effect of cues left behind by maggots and implications in their aggregation. *Scientific reports, 8*(1), 2844.

Gallagher, M. B., Sandhu, S., & Kimsey, R. (2010). Variation in developmental time for geographically distinct populations of the common green bottle fly, Lucilia sericata (Meigen). *J Forensic Sci, 55*. doi:10.1111/j.1556-4029.2009.01285.x

García-Bellido, A., & de Celis, J. F. (2009). The complex tale of the achaete–scute complex: a paradigmatic case in the analysis of gene organization and function during development. *Genetics, 182*(3), 631-639.

Geberth, V. J. (1996). *Practical homicide investigation*. Boca Raton, FL: CRC Press.

Gibbens, Y. Y., Warren, J. T., Gilbert, L. I., & O'Connor, M. B. (2011). Neuroendocrine regulation of Drosophila metamorphosis requires TGFβ/Activin signaling. *Development, 138*(13), 2693-2703. doi:10.1242/dev.063412

Gibbens, Y. Y., Warren, J. T., Gilbert, L. I., & O'Connor, M. B. (2011). Neuroendocrine regulation of Drosophila metamorphosis requires TGFβ/Activin signaling. *Development, 138*(13), 2693-2703.

Gilbert, L. I. (2004). Halloween genes encode P450 enzymes that mediate steroid hormone biosynthesis in Drosophila melanogaster. *Molecular and cellular endocrinology, 215*(1-2), 1-10.

Gilbert, L. I. (2008). Drosophila is an inclusive model for human diseases, growth and development. *Molecular and cellular endocrinology, 293*(1-2), 25-31.

Gill, H. K., Goyal, G., & Chahil, G. Insect Diapause: A Review.

Goff, M. L., Charbonneau, S., & Sullivan, W. (1991). Presence of fecal material in diapers as a potential source of error in estimations of postmortem interval using arthropod development rates. *Journal of Forensic Science, 36*(5), 1603-1606.

Goff, M. L., & Lord, W. D. (1994). Entomotoxicology: A New Area for Forensic Investigation. *The American journal of forensic medicine and pathology, 15*(1), 51-57.

Goodbrod, J. R., & Goff, M. L. (1990). Effects of larval population density on rates of development and interactions between two species of Chrysomya (Diptera: Calliphoridae) in laboratory culture. *J Med Entomol, 27*(3), 338-343.

Gramates, L. S., Marygold, S. J., Santos, G. d., Urbano, J.-M., Antonazzo, G., Matthews, B. B., . . . Zhou, P. (2017). FlyBase at 25: looking to the future. *Nucleic Acids Research, 45*(D1), D663-D671. doi:10.1093/nar/gkw1016

Greenberg, B. (1991). Flies as forensic indicators. *J Med Entomol, 28*(5), 565-577.

Gruner, S. V., Slone, D. H., & Capinera, J. L. (2007). Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. *Journal of Medical Entomology, 44*(3), 509-515.

Hall, T. A. (1999). *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.* Paper presented at the Nucleic acids symposium series.

Hannemann, F., Bichet, A., Ewen, K. M., & Bernhardt, R. (2007). Cytochrome P450 systems—biological variations of electron transport chains. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1770*(3), 330-344.

Heard, S. B., & Remer, L. C. (1997). Clutch-size behavior and coexistence in ephemeralpatch competition models. *The American Naturalist, 150*(6), 744-770.

Henssge, C., & Knight, B. (1995). *The Estimation of the Time Since Death in the Early Postmortem Period*: Edward Arnold.

Hynes, R. O., & Zhao, Q. (2000). The evolution of cell adhesion. *The Journal of cell biology, 150*(2), F89-F96.

Introna, F., Altamura, B., Dell, Erba, A., & Dattoli, V. (1989). Time Since Death Definition by Experimental Reproduction of Lucilia Sericata Cycles in Growth Cabinet.

James, M. T. (1947). *The Flies that Cause Myiasis in Man*: U.S. Department of Agriculture.

Jha, A. R., Miles, C. M., Lippert, N. R., Brown, C. D., White, K. P., & Kreitman, M. (2015). Whole-Genome Resequencing of Experimental Populations Reveals Polygenic Basis of Egg-Size Variation in Drosophila melanogaster. *Mol Biol Evol, 32*(10), 2616-2632. doi:10.1093/molbev/msv136

Johnson, M. D. (1975). Seasonal and microseral variations in the insect populations on carrion. *American Midland Naturalist*, 79-90.

Joseph, I., Mathew, D. G., Sathyan, P., & Vargheese, G. (2011). The use of insects in forensic investigations: An overview on the scope of forensic entomology. *J Forensic Dent Sci, 3*(2), 89-91. doi:10.4103/0975-1475.92154

Kadonaga, J. T. (2004). Regulation of RNA polymerase II transcription by sequence-specific DNA binding factors. *Cell, 116*(2), 247-257.

Kaiser, A., Klok, C. J., Socha, J. J., Lee, W.-K., Quinlan, M. C., & Harrison, J. F. (2007). Increase in tracheal investment with beetle size supports hypothesis of oxygen limitation on insect gigantism. *Proceedings of the National Academy of Sciences, 104*(32), 13198-13203.

Kim, D. H., Sarbassov, D. D., Ali, S. M., King, J. E., Latek, R. R., Erdjument-Bromage, H., . . . Sabatini, D. M. (2002). mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. *Cell, 110*(2), 163-175.

Kofler, R., Pandey, R. V., & Schlotterer, C. (2011). PoPoolation2: identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). *Bioinformatics, 27*(24), 3435-3436. doi:10.1093/bioinformatics/btr589

Kondo, M., Yamaoka, T., Honda, S., Miwa, Y., Katashima, R., Moritani, M., . . . Itakura, M. (2000). The rate of cell growth is regulated by purine biosynthesis via ATP production and G1 to S phase transition. *The Journal of Biochemistry, 128*(1), 57-64.

Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics, 23*(10), 1289-1291. doi:10.1093/bioinformatics/btm091

Krafsur, E., Whitten, C., & Novy, J. (1987). Screwworm eradication in north and central America. *Parasitology Today, 3*(5), 131-137.

Layalle, S., Arquier, N., & Léopold, P. (2008). The TOR pathway couples nutrition and developmental timing in Drosophila. *Developmental Cell, 15*(4), 568-577.

Lecheta, M. C., Thyssen, P. J., & Moura, M. O. (2015). The effect of temperature on development of Sarconesia chlorogaster, a blowfly of forensic importance. *Forensic science, medicine, and pathology, 11*(4), 538-543.

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics, 27*(21), 2987-2993. doi:10.1093/bioinformatics/btr509

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics, 26*(5), 589-595. doi:10.1093/bioinformatics/btp698

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., . . . Genome Project Data Processing, S. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*(16), 2078-2079. doi:10.1093/bioinformatics/btp352

Li, T., & Bender, M. (2000). A conditional rescue system reveals essential functions for the ecdysone receptor (EcR) gene during molting and metamorphosis in Drosophila. *Development, 127*(13), 2897-2905.

Massagué, J., & Chen, Y.-G. (2000). Controlling TGF-β signaling. *Genes & development, 14*(6), 627-644.

McBrayer, Z., Ono, H., Shimell, M., Parvy, J.-P., Beckstead, R. B., Warren, J. T., . . . O'Connor, M. B. (2007). Prothoracicotropic hormone regulates developmental timing and body size in Drosophila. *Developmental Cell, 13*(6), 857-871.

McWatters, H., & Saunders, D. (1997). Inheritance of the photoperiodic response controlling larval diapause in the blow fly, Calliphora vicina. *Journal of insect physiology, 43*(8), 709-717.

McWilliam, H., Li, W., Uludag, M., Squizzato, S., Park, Y. M., Buso, N., . . . Lopez, R. (2013). Analysis Tool Web Services from the EMBL-EBI. *Nucleic Acids Research, 41*(W1), W597-W600. doi:10.1093/nar/gkt376

Miloloza, A., Rosner, M., Nellist, M., Halley, D., Bernaschek, G., & Hengstschlager, M. (2000). The TSC1 gene product, hamartin, negatively regulates cell proliferation. *Hum Mol Genet, 9*(12), 1721-1727.

Mondor, E., Tremblay, M., Tomberlin, J., Benbow, E., Tarone, A., & Crippen, T. (2012). The ecology of carrion decomposition. *Nat Educ Knowledge, 3*, 21.

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schaffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics, 24*(16), 1757-1764. doi:10.1093/bioinformatics/btn322

Nabity, P. D., Higley, L. G., & Heng-Moss, T. M. (2006). Effects of temperature on development of Phormia regina (Diptera: Calliphoridae) and use of developmental data in determining time intervals in forensic entomology. *J Med Entomol, 43*(6), 1276-1286. doi:10.1603/0022-2585(2006)43[1276:EOTODO]2.0.CO;2

Negre, B., & Simpson, P. (2015). The achaete-scute complex in Diptera: patterns of noncoding sequence evolution. *J Evol Biol, 28*. doi:10.1111/jeb.12687

Nijhout, H. F., & Williams, C. M. (1974a). Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta (L.): cessation of juvenile hormone secretion as a trigger for pupation. *Journal of Experimental Biology, 61*(2), 493-501.

Nijhout, H. F., & Williams, C. M. (1974b). Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta (L.): growth of the last-instar larva and the decision to pupate. *Journal of Experimental Biology, 61*(2), 481-491.

Novy, J. E. (1991). Screwworm control and eradication in the southern United States of America. *World Animal Review, 10*, 18-27.

Núñez–Vázquez, C., Tomberlin, J. K., Cantú–Sifuentes, M., & García–Martínez, O. (2013). Laboratory development and field validation of Phormia regina (Diptera: Calliphoridae). *Journal of Medical Entomology, 50*(2), 252-260.

Nunney, L. (1996). The response to selection for fast larval development in Drosophila melanogaster and its effect on adult weight: an example of a fitness trade-off. *Evolution, 50*(3), 1193-1204.

Nuzhdin, S. V., Harshman, L. G., Zhou, M., & Harmon, K. (2007). Genome-enabled hitchhiking mapping identifies QTLs for stress resistance in natural Drosophila. *Heredity, 99*(3), 313.

Nuzhdin, S. V., Pasyukova, E. G., Dilda, C. L., Zeng, Z.-B., & Mackay, T. F. (1997). Sex-specific quantitative trait loci affecting longevity in Drosophila melanogaster. *Proceedings of the National Academy of Sciences, 94*(18), 9734-9739.

Oliveira, H. G., Gomes, G., Morlin Jr, J. J., Von Zuben, C. J., & Linhares, A. X. (2009). The effect of Buscopan® on the development of the blow fly Chrysomya megacephala (F.)(Diptera: Calliphoridae). *Journal of forensic sciences, 54*(1), 202-206.

Orozco-terWengel, P., Kapun, M., Nolte, V., Kofler, R., Flatt, T., & Schlotterer, C. (2012). Adaptation of Drosophila to a novel laboratory environment reveals temporally heterogeneous trajectories of selected alleles. *Mol Ecol, 21*(20), 4931-4941. doi:10.1111/j.1365-294X.2012.05673.x

Owings, C. (2014). Developmental variation among Cochliomyia macellaria Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. *International Journal of Legal Medicine, 128*, 9.

Owings, C. G., Spiegelman, C., Tarone, A. M., & Tomberlin, J. K. (2014). Developmental variation among Cochliomyia macellaria Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. *International Journal of Legal Medicine, 128*(4), 709-717.

Parvy, J.-P., Blais, C., Bernard, F., Warren, J., Petryk, A., Gilbert, L., . . . Dauphin-Villemant, C. (2005). A role for βFTZ-F1 in regulating ecdysteroid titers during post-embryonic development in Drosophila melanogaster. *Developmental Biology, 282*(1), 84-94.

Pazman, C., Mayes, C. A., Fanto, M., Haynes, S. R., & Mlodzik, M. (2000). Rasputin, the Drosophila homologue of the RasGAP SH3 binding protein, functions in ras- and Rho-mediated signaling. *Development, 127*(8), 1715-1725.

Peterson, A. J., Jensen, P. A., Shimell, M., Stefancsik, R., Wijayatonge, R., Herder, R., . . . O'Connor, M. B. (2012). R-Smad competition controls activin receptor output in Drosophila. *PLoS One, 7*(5), e36548. doi:10.1371/journal.pone.0036548

Pokholok, D. K., Zeitlinger, J., Hannett, N. M., Reynolds, D. B., & Young, R. A. (2006). Activated signal transduction kinases frequently occupy target genes. *Science, 313*(5786), 533-536.

Putman, R. (1983). *Carrion and Dung: The Decomposition of Animal Wastes*: Edward Arnold.

Ramos III, E. (2015). *A Genetic Study of the Development of Cochliomyia macellaria (Fabricius)(Diptera: Calliphoridae): Ecological, Evolutionary, and Forensic Importance of the Secondary Screwworm.*

Reed, L. K., Lee, K., Zhang, Z., Rashid, L., Poe, A., Hsieh, B., . . . Gibson, G. (2014). Systems genomics of metabolic phenotypes in wild-type Drosophila melanogaster. *Genetics, 197*(2), 781-793. doi:10.1534/genetics.114.163857

Reigada, C., & Godoy, W. (2006). Larval density, temperature and biological aspects of Chrysomya megacephala (Diptera: Calliphoridae). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 58*(4), 562-566.

Remolina, S. C., Chang, P. L., Leips, J., Nuzhdin, S. V., & Hughes, K. A. (2012). Genomic basis of aging and life-history evolution in Drosophila melanogaster. *Evolution, 66*(11), 3390-3403. doi:10.1111/j.1558-5646.2012.01710.x

Rewitz, K. F., Yamanaka, N., Gilbert, L. I., & O'Connor, M. B. (2009). The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. *Science, 326*(5958), 1403-1405.

Rewitz, K. F., Yamanaka, N., & O'Connor, M. B. (2013). Developmental checkpoints and feedback circuits time insect maturation *Current topics in developmental biology* (Vol. 103, pp. 1-33): Elsevier.

Sakurai, S. (1984). Temporal organization of endocrine events underlying larval-pupal metamorphosis in the silkworm, Bombyx mori. *Journal of insect physiology, 30*(8), 657-664.

Schoenly, K. (1992). A statistical analysis of successional patterns in carrion-arthropod assemblages: implications for forensic entomology and determination of the postmortem interval. *J Forensic Sci, 37*(6), 1489-1513.

Schoenly, K., & Reid, W. (1987). Dynamics of heterotrophic succession in carrion arthropod assemblages: discrete seres or a continuum of change? *Oecologia, 73*(2), 192-202. doi:10.1007/BF00377507

Schröder, M., Giermann, N., & Zrenner, R. (2005). Functional analysis of the pyrimidine de novo synthesis pathway in solanaceous species. *Plant physiology, 138*(4), 1926-1938.

Shingleton, A. W., Das, J., Vinicius, L., & Stern, D. L. (2005). The temporal requirements for insulin signaling during development in Drosophila. *PLoS biology, 3*(9), e289.

Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., . . . Higgins, D. G. (2011). Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol, 7*, 539. doi:10.1038/msb.2011.75

Stanke, M., Steinkamp, R., Waack, S., & Morgenstern, B. (2004). AUGUSTUS: a web server for gene finding in eukaryotes. *Nucleic Acids Res, 32*(Web Server issue), W309-312. doi:10.1093/nar/gkh379

Tarone, A. a. F., David. (2006). Components of Developmental Plasticity in a Michigan Population of Lucilia sericata (Diptera Calliphoridae). *Journal of Medical Entomology, 43*(5), 11.

Tarone, A. M., & Foran, D. R. (2006). Components of developmental plasticity in a Michigan population of Lucilia sericata (Diptera: Calliphoridae). *J Med Entomol, 43*(5), 1023-1033.

Tarone, A. M., Picard, C., & Sze, S.-H. (2016). *Genomic Tools to Reduce Error in PMI Estimates Derived from Entomological Evidence*: Texas A&M Research Foundation.

Tarone, A. M., Picard, C. J., Spiegelman, C., & Foran, D. R. (2011). Population and Temperature Effects on Lucilia sericata (Diptera: Calliphoridae) Body Size and Minimum Development Time. *J Med Entomol, 48*. doi:10.1603/me11004

Thummel, C. S. (1996). Flies on steroids—Drosophila metamorphosis and the mechanisms of steroid hormone action. *Trends in Genetics, 12*(8), 306-310.

Thummel, C. S. (2001). Molecular mechanisms of developmental timing in C. elegans and Drosophila. *Dev Cell, 1*(4), 453-465.

Thyssen, P. J., Nassu, M. P., Costella, A. M. U., & Costella, M. L. (2012). Record of oral myiasis by Cochliomyia hominivorax (Diptera: Calliphoridae): case evidencing negligence in the treatment of incapable. *Parasitology Research, 111*(2), 957-959.

Tijet, N., Helvig, C., & Feyereisen, R. (2001). The cytochrome P450 gene superfamily in Drosophila melanogaster: annotation, intron-exon organization and phylogeny. *Gene, 262*(1), 189-198.

Tobler, R., Franssen, S. U., Kofler, R., Orozco-Terwengel, P., Nolte, V., Hermisson, J., & Schlotterer, C. (2014). Massive habitat-specific genomic response in D. melanogaster populations during experimental evolution in hot and cold environments. *Mol Biol Evol, 31*(2), 364-375. doi:10.1093/molbev/mst205

Turner, B., & Howard, T. (1992). Metabolic heat generation in dipteran larval aggregations: a consideration for forensic entomology. *Medical and veterinary entomology, 6*(2), 179-181.

Turner, T. L., & Miller, P. M. (2012). Investigating natural variation in Drosophila courtship song by the evolve and resequence approach. *Genetics, 191*(2), 633-642. doi:10.1534/genetics.112.139337

Turner, T. L., Stewart, A. D., Fields, A. T., Rice, W. R., & Tarone, A. M. (2011). Population-Based Resequencing of Experimentally Evolved Populations Reveals the Genetic Basis of Body Size Variation in Drosophila melanogaster. *PLOS Genetics, 7*(3), e1001336. doi:10.1371/journal.pgen.1001336

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Research, 40*(15), e115-e115. doi:10.1093/nar/gks596

Van Obberghen, E., Ksauga, M., Le Cam, A., Hedo, J. A., Itin, A., & Harrison, L. C. (1981). Biosynthetic labeling of insulin receptor: studies of subunits in cultured human IM-9 lymphocytes. *Proceedings of the National Academy of Sciences, 78*(2), 1052-1056.

Wells, J. D., & Greenberg, B. (1992). Laboratory Interaction Between Introduced Chrysomya rufifacies and Native Cochliomyia macellaria (Diptera: Calliphoridae). *Environmental Entomology, 21*(3), 640-645. doi:10.1093/ee/21.3.640

Whitworth, T. (2006). Keys to the genera and species of blow flies (Diptera : Calliphoridae) of America North of Mexico. *Proc Entomol Soc Wash, 108*.

Yamanaka, N., Rewitz, K. F., & O'Connor, M. B. (2013). Ecdysone control of developmental transitions: lessons from Drosophila research. *Annu Rev Entomol, 58*, 497-516. doi:10.1146/annurev-ento-120811-153608

Yang, S.-T., & Shiao, S.-F. (2012). Oviposition preferences of two forensically important blow fly species, Chrysomya megacephala and C. rufifacies (Diptera: Calliphoridae), and implications for postmortem interval estimation. *Journal of Medical Entomology, 49*(2), 424-435.

Zhou, D., Udpa, N., Gersten, M., Visk, D. W., Bashir, A., Xue, J., . . . Haddad, G. G. (2011). Experimental selection of hypoxia-tolerant Drosophila melanogaster. *Proc Natl Acad Sci U S A, 108*(6), 2349-2354. doi:10.1073/pnas.1010643108

Ziffren, S. E., Heist, H. E., May, S. C., & Womack, N. A. (1953). The secretion of collagenase by maggots and its implication. *Annals of surgery, 138*(6), 932.

Zumpt, F. (1965). *Myiasis in Man and Animals in the Old World: A Textbook for Physicians, Veterinarians, and Zoologists*: Butterworths.

APPENDIX A

Popoolation2 script for identifying consistent allele frequency changes in two biological replicates.

Modules required to run the Popoolation2 scripts include: BWA v0.7.12, GCC v6.3.0, SAMTools v1.5, Java v1.8.0_131, Perl v5.24.1, and R v3.3.1.

Example populations: pop1 is fast developing replicate one, pop2 is slow developing replicate one, pop3 is fast developing replicate two, and pop4 is slow developing replicate 2.

```
#Indexing of Reference Genome

bwa index Reference.fa

#BWA mapping of trimmed paired reads for each geographic replicate to the Reference Genome

bwa mem -t $P -aM Reference.fa pop1_trim.fastq > pop1.sam

bwa mem -t $P -aM Reference.fa pop2_trim.fastq > pop2.sam

bwa mem -t $P -aM Reference.fa pop3_trim.fastq > pop3.sam

bwa mem -t $P -aM Reference.fa pop4_trim.fastq > pop4.sam

#Remove ambiguous reads and formatting into binary alignment file

samtools view -Shu pop1.sam | samtools sort > pop1.bam

samtools view -Shu pop2.sam | samtools sort > pop2.bam

samtools view -Shu pop3.sam | samtools sort > pop3.bam
```

samtools view -Shu pop4.sam | samtools sort > pop4.bam

#Create a synchronized file

samtools mpileup -B -f Reference.fa pop1.bam pop2.bam pop3.bam pop4.bam > pop1_pop2_pop3_pop4.mpileup

java -ea -Xmx2g -jar ~/popoolation2_1201/mpileup2sync.jar --input pop1_pop2_pop3_pop4.mpileup --output pop1_pop2_pop3_pop4.sync --fastq-type sanger --min-qual 2 --threads 12

#Detect consistent allele frequency changes in two biological replicates using Cochran-Mantel-Haenszel test.

perl ~/popoolation2_1201/cmh-test.pl –input pop1_pop2_pop3_pop4.sync --output pop1_pop2_pop3_pop4.cmh --max-coverage 2% --population 1-2,3-4

APPENDIX B

Identifying significant variants calls from the Popoolation2 Cochran-Mantel-Haenszel test using the Bioconductor Q-Value Package.

RStudio Script

#Open the file as a new data frame in RStudio

File <- read.delim("C:~/cmh.csv", header=FALSE)

#Load the Q-Value Package

library(qvalue)

#Estimate the *Q* values from the *P* values in the eighth column while applying a False Discovery Rate (for this experiment 0.1% FDR was used)

qobj<-qvalue(File$V8, fdr.level=0.001)

#Calculate the number of "TRUE," or significant, variants

table(qobj$significant)["TRUE"]

#Add a new column containing the *q* values to the original data frame

File$V9<-qobj$qvalues

#Add a new column containing the "TRUE" and "FALSE" calls for significant variants to the original data frame

File$V10<-qobj$qvalues<0.001

```
#Create a new data table containing the significant variant data

File2 <- subset(File, V10 == "TRUE")

#Identify the number of contigs containing significant variants

length(unique(File2$V1))

#Load data table library

library(data.table)

#Create frequency column with the number of times a contig name is repeated, reports the number of variants in each contig

setDT(File2)[,freq := .N, by = c("V1")]

#Sort the frequency column by decreasing value

File2[order(freq, decreasing = T),]

#Make new data frame sorted by decreasing frequency values

File3 <- File2[order(-freq)]

#Create a list of sorted contigs

File4 <- File3$V1

#Remove duplicate contig names leaving a list of contigs. This creates the list on contigs containing significant variants

File5 <- File4[!duplicated(File4)]
```

```
#Export data frame into a text document

write.table(File, "c:/File.txt", sep="\t")
```

APPENDIX C

BLAST results from the 699 sequences containing significant variants

Table 4. The BLAST results from the 699 *C. macellaria* sequences and associated E Values. E values is the probability that the blast hit was random, or by chance.

C. macellaria Sequence	Description of Blast Results	e-Value
A26S_Cmac_wdnm_contig_10076	N/A	
A26S_Cmac_wdnm_contig_1008	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_10132	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.67E-07
A26S_Cmac_wdnm_contig_10152	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.07E-101
A26S_Cmac_wdnm_contig_10159	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	2.90E-26
A26S_Cmac_wdnm_contig_10216	N/A	
A26S_Cmac_wdnm_contig_10319	*Calliphora vicina* Achaete-Scute clone BAC 97L04	1.28E-28
A26S_Cmac_wdnm_contig_10353	*Cochliomyia macellaria* clone Cmac8 LINE CR1 sequence	1.38E-42
A26S_Cmac_wdnm_contig_10375	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	5.09E-32
A26S_Cmac_wdnm_contig_10392	*Drosophila willistoni* alan shepard (LOC6639197) transcript variant mRNA	3.27E-151
A26S_Cmac_wdnm_contig_10397	*Plutella xylostella* probable transcriptional regulator ycf27 (LOC105397525) mRNA	7.03E-81
A26S_Cmac_wdnm_contig_1048	*Lucilia cuprina* angiotensin-converting enzyme-like (LOC111678189) mRNA	5.02E-110
A26S_Cmac_wdnm_contig_10494	N/A	
A26S_Cmac_wdnm_contig_10538	*Calliphora vicina* Achaete-Scute clone BAC 99M22	5.70E-73
A26S_Cmac_wdnm_contig_10583	N/A	
A26S_Cmac_wdnm_contig_10599	*Calliphora vicina* Achaete-Scute clone BAC 99M22	2.61E-21
A26S_Cmac_wdnm_contig_10703	*Rhagoletis zephyria* uncharacterized LOC108373869 (LOC108373869) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_10711	*Drosophila takahashii* diacylglycerol kinase 1 (LOC108067820) transcript variant mRNA	2.06E-43
A26S_Cmac_wdnm_contig_10732	*Cochliomyia macellaria* transformer (tra) complete cds	7.70E-33
A26S_Cmac_wdnm_contig_10783	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	7.06E-06
A26S_Cmac_wdnm_contig_108	*Plutella xylostella* acyl- dehydrogenase family member mitochondrial-like (LOC105397323) mRNA	1.83E-177
A26S_Cmac_wdnm_contig_1080	*Musca domestica* ATP synthase subunit mitochondrial (LOC101901126) transcript variant mRNA	0

Table 4. continued

A26S_Cmac_wdnm_contig_10812	*Lucilia cuprina* phosphoserine phosphatase-like (LOC111685524) mRNA	5.96E-138
A26S_Cmac_wdnm_contig_10852	*Ceratina calcarata* elongation factor Tu-like (LOC108624706) mRNA	2.37E-69
A26S_Cmac_wdnm_contig_1090	*Agrilus planipennis* GDP-mannose 4,6 dehydratase-like (LOC108741065) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_10919	*Lucilia cuprina* RNA chaperone -like (LOC111681694) mRNA	1.15E-123
A26S_Cmac_wdnm_contig_11147	*Gryllus bimaculatus* GBcontig01632	1.24E-07
A26S_Cmac_wdnm_contig_1121	*Lucilia cuprina* probable L-xylulose kinase (LOC111685616) mRNA	0
A26S_Cmac_wdnm_contig_11222	*Musca domestica* mediator of RNA polymerase II transcription subunit 26 (LOC101898239) transcript variant mRNA	2.85E-55
A26S_Cmac_wdnm_contig_11344	*Lucilia cuprina* DNA polymerase III subunit tau-like (LOC111678789) mRNA	0
A26S_Cmac_wdnm_contig_11496	*Calliphora vicina* Achaete-Scute clone BAC 62B24	2.72E-52
A26S_Cmac_wdnm_contig_11503	N/A	
A26S_Cmac_wdnm_contig_11514	*Lucilia cuprina* probable ubiquitin carboxyl-terminal hydrolase FAF (LOC111676552) transcript variant mRNA	4.60E-26
A26S_Cmac_wdnm_contig_11550	N/A	
A26S_Cmac_wdnm_contig_11594	*Bactrocera dorsalis* vacuolar sorting-associated 13D (LOC105230284) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_1161	*Lucilia cuprina* transport Sec24A (LOC111675420) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_11615	*Cochliomyia macellaria* transformer (tra) complete cds	1.06E-11
A26S_Cmac_wdnm_contig_11731	*Lucilia cuprina* 30S ribosomal S3 (LOC111689111) mRNA	0
A26S_Cmac_wdnm_contig_11806	*Lucilia cuprina* yolk D (ypD) cds and yolk A (ypA) and yolk B (ypB) complete cds	1.08E-12
A26S_Cmac_wdnm_contig_1187	*Lucilia cuprina* extracellular serine protease-like (LOC111685027) mRNA	0
A26S_Cmac_wdnm_contig_12045	*Lucilia cuprina* uncharacterized transporter -like (LOC111682663) mRNA	0
A26S_Cmac_wdnm_contig_12098	N/A	
A26S_Cmac_wdnm_contig_12111	*Bactrocera oleae* structural maintenance of chromosomes 5 (LOC106616819) transcript variant mRNA	6.67E-80
A26S_Cmac_wdnm_contig_12231	N/A	
A26S_Cmac_wdnm_contig_12259	*Drosophila melanogaster* clone complete sequence	4.42E-18

Table 4. continued

A26S_Cmac_wdnm_contig_123	*Lucilia cuprina* primosomal N -like (LOC111689852) mRNA	0
A26S_Cmac_wdnm_contig_12331	*Acromyrmex echinatior* uncharacterized LOC105145247 (LOC105145247) transcript variant ncRNA	3.33E-26
A26S_Cmac_wdnm_contig_1235	N/A	
A26S_Cmac_wdnm_contig_12400	*Lucilia cuprina* phosphatidylinositol transfer beta isoform (LOC111688196) transcript variant mRNA	3.25E-90
A26S_Cmac_wdnm_contig_12417	*Cochliomyia macellaria* transformer (tra) complete cds	4.89E-22
A26S_Cmac_wdnm_contig_1245	*Diachasma alloeum* DNA-directed RNA polymerase subunit beta (LOC107047206) mRNA	0
A26S_Cmac_wdnm_contig_12495	*Musca domestica* uncharacterized LOC109612107 (LOC109612107) mRNA	4.77E-34
A26S_Cmac_wdnm_contig_12527	*Drosophila miranda* strain MSH22 chromosome 4 clone complete sequence	1.92E-39
A26S_Cmac_wdnm_contig_1253	*Lucilia cuprina* probable isoaspartyl peptidase L-asparaginase CG7860 (LOC111679294) mRNA	1.30E-08
A26S_Cmac_wdnm_contig_12566	*Lucilia cuprina* dedicator of cytokinesis 1 (LOC111690482) transcript variant mRNA	8.53E-26
A26S_Cmac_wdnm_contig_1271	*Nasonia vitripennis* type I restriction enzyme R (LOC107981567) mRNA	0
A26S_Cmac_wdnm_contig_12742	N/A	
A26S_Cmac_wdnm_contig_12746	*Cochliomyia macellaria* clone Cmac4 LINE CR1 sequence	1.31E-41
A26S_Cmac_wdnm_contig_12833	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	1.91E-110
A26S_Cmac_wdnm_contig_12851	N/A	
A26S_Cmac_wdnm_contig_12874	*Drosophila obscura* neuropeptide CCHamide-2 receptor (LOC111079801) transcript variant mRNA	1.33E-110
A26S_Cmac_wdnm_contig_12911	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.56E-172
A26S_Cmac_wdnm_contig_12934	*Culicoides sonorensis* genome scaffold: scaffold29	4.44E-19
A26S_Cmac_wdnm_contig_12937	*Ceratitis capitata* sodium potassium-transporting ATPase subunit beta-1-interacting (LOC101452082) transcript variant mRNA	6.00E-165
A26S_Cmac_wdnm_contig_12991	*Cochliomyia macellaria* transformer (tra) complete cds	6.29E-24
A26S_Cmac_wdnm_contig_13009	N/A	
A26S_Cmac_wdnm_contig_1304	*Lucilia cuprina* ferric transport system permease -like (LOC111689248) mRNA	0
A26S_Cmac_wdnm_contig_13103	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.30E-08
A26S_Cmac_wdnm_contig_13153	*Lucilia cuprina* uncharacterized LOC111689750 (LOC111689750) mRNA	1.75E-09

Table 4. continued

A26S_Cmac_wdnm_contig_13199	*Bactrocera latifrons* E3 ubiquitin- ligase UBR1-like (LOC108970669) transcript variant mRNA	1.23E-126
A26S_Cmac_wdnm_contig_13239	*Bactrocera latifrons* melted (LOC108978472) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_1337	N/A	
A26S_Cmac_wdnm_contig_13470	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.52E-60
A26S_Cmac_wdnm_contig_13587	*Calliphora vicina* Achaete-Scute clone BAC 99M22	4.43E-90
A26S_Cmac_wdnm_contig_1363	*Lucilia cuprina* ferric transport system permease -like (LOC111689248) mRNA	0
A26S_Cmac_wdnm_contig_13705	*Bactrocera cucurbitae* endoribonuclease Dcr-1 (LOC105214300) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_1371		
A26S_Cmac_wdnm_contig_13730	N/A	
A26S_Cmac_wdnm_contig_1377		
A26S_Cmac_wdnm_contig_13945	*Cochliomyia macellaria* transformer (tra) complete cds	1.71E-08
A26S_Cmac_wdnm_contig_13959	*Rhagoletis zephyria* uncharacterized LOC108368723 (LOC108368723) transcript variant mRNA	2.69E-98
A26S_Cmac_wdnm_contig_13976	N/A	
A26S_Cmac_wdnm_contig_13980	*Plutella xylostella* uncharacterized LOC105395803 (LOC105395803) mRNA	0
A26S_Cmac_wdnm_contig_13994	*Lucilia cuprina* (LOC111682324) mRNA	3.30E-112
A26S_Cmac_wdnm_contig_14015	*Rhagoletis zephyria* XK-related 4-like (LOC108373676) transcript variant mRNA	1.26E-57
A26S_Cmac_wdnm_contig_14132	*Cochliomyia macellaria* transformer (tra) complete cds	2.96E-38
A26S_Cmac_wdnm_contig_14160	N/A	
A26S_Cmac_wdnm_contig_1417	*Nasonia vitripennis* 22 kDa relaxation -like (LOC107981719) mRNA	2.93E-130
A26S_Cmac_wdnm_contig_14170	*Lucilia cuprina* 3-hydroxyacyl-dehydrogenase type-2 (LOC111678909) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_1418	N/A	
A26S_Cmac_wdnm_contig_14243	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.30E-52
A26S_Cmac_wdnm_contig_14335	*Lucilia cuprina* HSP83 complete cds	1.58E-13
A26S_Cmac_wdnm_contig_14366	*Plutella xylostella* carbamoyl-phosphate synthase large chloroplastic (LOC105397066) mRNA	0
A26S_Cmac_wdnm_contig_14374	N/A	
A26S_Cmac_wdnm_contig_14381	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	5.24E-50
A26S_Cmac_wdnm_contig_14481	*Drosophila melanogaster* clone complete sequence	1.34E-91

Table 4. continued

A26S_Cmac_wdnm_contig_14618	N/A	
A26S_Cmac_wdnm_contig_14703	N/A	
A26S_Cmac_wdnm_contig_14800	*Stomoxys calcitrans* uncharacterized LOC106085956 (LOC106085956) transcript variant ncRNA	1.02E-11
A26S_Cmac_wdnm_contig_14847	*Lucilia cuprina* uncharacterized LOC111681958 (LOC111681958) mRNA	4.54E-25
A26S_Cmac_wdnm_contig_1489	N/A	
A26S_Cmac_wdnm_contig_14904	*Calliphora vicina* Achaete-Scute clone BAC 62B24	2.25E-06
A26S_Cmac_wdnm_contig_15081	N/A	
A26S_Cmac_wdnm_contig_15106	*Calliphora vicina* Achaete-Scute clone BAC 113H10	1.99E-09
A26S_Cmac_wdnm_contig_15113	*Drosophila melanogaster* clone complete sequence	0
A26S_Cmac_wdnm_contig_15170	N/A	
A26S_Cmac_wdnm_contig_15209	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.57E-26
A26S_Cmac_wdnm_contig_1522	*Plutella xylostella* uncharacterized LOC105396924 (LOC105396924) mRNA	0
A26S_Cmac_wdnm_contig_1523	*Gryllus bimaculatus* GBcontig00259	5.24E-10
A26S_Cmac_wdnm_contig_15245	*Drosophila busckii* serine threonine-kinase Doa (LOC108602793) transcript variant mRNA	3.05E-23
A26S_Cmac_wdnm_contig_1525	*Lucilia cuprina* glycine oxidase-like (LOC111688127) mRNA	0
A26S_Cmac_wdnm_contig_15260	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.38E-38
A26S_Cmac_wdnm_contig_15526	*Musca domestica* cytochrome c oxidase subunit mitochondrial (LOC101899299) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_1557		
A26S_Cmac_wdnm_contig_15606	*Lucilia cuprina* threonylcarbamoyl-AMP synthase-like (LOC111689117) mRNA	7.16E-40
A26S_Cmac_wdnm_contig_1566	N/A	
A26S_Cmac_wdnm_contig_15708	*Lucilia cuprina* high affinity cationic amino acid transporter 1-like (LOC111675499) mRNA	1.35E-10
A26S_Cmac_wdnm_contig_15785	*Bactrocera latifrons* ras-related Rab-26 (LOC108969216) transcript variant mRNA	1.56E-30
A26S_Cmac_wdnm_contig_15803	*Stomoxys calcitrans* uncharacterized LOC106087071 (LOC106087071) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_15915	*Cochliomyia macellaria* transformer (tra) complete cds	2.02E-17
A26S_Cmac_wdnm_contig_15987	*Cochliomyia macellaria* transformer (tra) complete cds	1.85E-06
A26S_Cmac_wdnm_contig_16019	N/A	
A26S_Cmac_wdnm_contig_16047	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_16098	*Ceratitis capitata* moesin ezrin radixin homolog 1 (LOC101457904) transcript variant mRNA	1.51E-162
A26S_Cmac_wdnm_contig_16143	N/A	
A26S_Cmac_wdnm_contig_1623	*Drosophila subobscura* map 12A chromosomal inversion A2 P275 region genomic sequence	4.43E-34
A26S_Cmac_wdnm_contig_16314	*Cochliomyia macellaria* transformer (tra) complete cds	2.45E-21
A26S_Cmac_wdnm_contig_16483	*Drosophila miranda* strain MSH22 chromosome 4 clone complete sequence	6.75E-29
A26S_Cmac_wdnm_contig_16484	*Cochliomyia macellaria* transformer (tra) complete cds	2.74E-19
A26S_Cmac_wdnm_contig_16704	N/A	
A26S_Cmac_wdnm_contig_16754	*Stomoxys calcitrans* kinesin Klp10A (LOC106085431) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_16767	*Drosophila melanogaster* clone complete sequence	0
A26S_Cmac_wdnm_contig_16809	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.57E-16
A26S_Cmac_wdnm_contig_16906	N/A	
A26S_Cmac_wdnm_contig_16930	*Drosophila busckii* chromosome 2L sequence	1.60E-30
A26S_Cmac_wdnm_contig_16984	N/A	
A26S_Cmac_wdnm_contig_17005	*Lucilia cuprina* organic cation transporter -like (LOC111684088) mRNA	5.15E-94
A26S_Cmac_wdnm_contig_17071	N/A	
A26S_Cmac_wdnm_contig_1710	*Lucilia cuprina* chemotaxis -like (LOC111676261) mRNA	1.38E-111
A26S_Cmac_wdnm_contig_17114	*Cochliomyia macellaria* transformer (tra) complete cds	3.55E-28
A26S_Cmac_wdnm_contig_17254	*Calliphora vicina* Achaete-Scute clone BAC 99M22	2.76E-33
A26S_Cmac_wdnm_contig_17396	*Lucilia cuprina* lap4 (LOC111674587) transcript variant mRNA	3.58E-65
A26S_Cmac_wdnm_contig_17399	N/A	
A26S_Cmac_wdnm_contig_17457	N/A	
A26S_Cmac_wdnm_contig_17486	*Calliphora vicina* Achaete-Scute clone BAC 99M22	4.82E-16
A26S_Cmac_wdnm_contig_17570	*Lucilia cuprina* aconitate hydratase B-like (LOC111679623) mRNA	0
A26S_Cmac_wdnm_contig_17608	*Lucilia cuprina* amidohydrolase (LOC111674675) mRNA	0
A26S_Cmac_wdnm_contig_17613	*Stomoxys calcitrans* uncharacterized LOC106085575 (LOC106085575) transcript variant ncRNA	2.31E-48
A26S_Cmac_wdnm_contig_17639	*Musca domestica* transmembrane 9 superfamily member 2 (LOC101901087) transcript variant mRNA	0

Table 4. continued

A26S_Cmac_wdnm_contig_17706	*Cochliomyia macellaria* transformer (tra) complete cds	3.15E-12
A26S_Cmac_wdnm_contig_17710	*Drosophila melanogaster* clone complete sequence	3.52E-10
A26S_Cmac_wdnm_contig_17775	*Cochliomyia macellaria* transformer (tra) complete cds	1.12E-22
A26S_Cmac_wdnm_contig_17860	*Cochliomyia macellaria* transformer (tra) complete cds	3.21E-14
A26S_Cmac_wdnm_contig_17956	*Lucilia cuprina* probable zinc protease (LOC111685518) mRNA	0
A26S_Cmac_wdnm_contig_17984	N/A	
A26S_Cmac_wdnm_contig_1801	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.29E-18
A26S_Cmac_wdnm_contig_1802	*Lucilia cuprina* porin B-like (LOC111674701) mRNA	0
A26S_Cmac_wdnm_contig_18031	N/A	
A26S_Cmac_wdnm_contig_18070	N/A	
A26S_Cmac_wdnm_contig_18083	*Cochliomyia macellaria* transformer (tra) complete cds	3.08E-61
A26S_Cmac_wdnm_contig_1816	*Nasonia vitripennis* succinate-semialdehyde dehydrogenase	0
A26S_Cmac_wdnm_contig_1817	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_18265	*Cochliomyia macellaria* transformer (tra) complete cds	6.72E-29
A26S_Cmac_wdnm_contig_18332	*Papilio maraho* isolate Pa1-171 complete genome	5.45E-07
A26S_Cmac_wdnm_contig_18440	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.91E-18
A26S_Cmac_wdnm_contig_18485	N/A	
A26S_Cmac_wdnm_contig_185	*Lucilia cuprina* aldehyde dehydrogenase (LOC111676707) mRNA	0
A26S_Cmac_wdnm_contig_18531	*Drosophila arizonae* geranylgeranyl transferase type-2 subunit alpha (LOC108609184) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_18557	N/A	
A26S_Cmac_wdnm_contig_18589	*Drosophila biarmipes* guanylate cyclase 32E (LOC108033304) transcript variant mRNA	3.39E-54
A26S_Cmac_wdnm_contig_18646	*Cochliomyia macellaria* transformer (tra) complete cds	2.30E-53
A26S_Cmac_wdnm_contig_18647	*Lucilia cuprina* penicillin-binding 1B-like (LOC111685745) mRNA	0
A26S_Cmac_wdnm_contig_1865	*Stomoxys calcitrans* dystrobrevin beta (LOC106080927) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_18732	*Cochliomyia macellaria* transformer (tra) complete cds	1.61E-23
A26S_Cmac_wdnm_contig_18780	N/A	
A26S_Cmac_wdnm_contig_1879	N/A	
A26S_Cmac_wdnm_contig_18880	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_18891	*Ceratitis capitata* ADP-ribosylation factor 2 (LOC101454075) transcript variant mRNA	1.17E-30
A26S_Cmac_wdnm_contig_18930	N/A	
A26S_Cmac_wdnm_contig_18978	*Lucilia cuprina* -like (LOC111689119) mRNA	3.54E-38
A26S_Cmac_wdnm_contig_1909	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_19095	N/A	
A26S_Cmac_wdnm_contig_19164	N/A	
A26S_Cmac_wdnm_contig_19187	*Lucilia cuprina* zinc finger 846-like (LOC111685543) mRNA	3.86E-10
A26S_Cmac_wdnm_contig_1920	*Lucilia cuprina* -like (LOC111683529) mRNA	6.69E-145
A26S_Cmac_wdnm_contig_19360	*Lucilia cuprina* alcohol dehydrogenase-like (LOC111682853) mRNA	3.45E-90
A26S_Cmac_wdnm_contig_19411	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.04E-44
A26S_Cmac_wdnm_contig_19480	*Gryllus bimaculatus* GBcontig28905	2.95E-151
A26S_Cmac_wdnm_contig_19492	*Lucilia cuprina* uncharacterized LOC111690014 (LOC111690014) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_195	*Plutella xylostella* carbamoyl-phosphate synthase large chloroplastic (LOC105397066) mRNA	0
A26S_Cmac_wdnm_contig_19505	N/A	
A26S_Cmac_wdnm_contig_19590	*Eurytemora affinis* uncharacterized LOC111699034 (LOC111699034) transcript variant mRNA	6.13E-22
A26S_Cmac_wdnm_contig_1971	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_19794	*Lucilia cuprina* sorbin and SH3 domain-containing 1 (LOC111691266) transcript variant mRNA	7.49E-129
A26S_Cmac_wdnm_contig_19960	*Calliphora vicina* Achaete-Scute clone BAC 97L04	3.10E-15
A26S_Cmac_wdnm_contig_20	*Lucilia cuprina* probable zinc protease (LOC111679944) mRNA	0
A26S_Cmac_wdnm_contig_20045	*Stomoxys calcitrans* uncharacterized LOC106085956 (LOC106085956) transcript variant ncRNA	5.00E-34
A26S_Cmac_wdnm_contig_20050	*Calliphora vicina* Achaete-Scute clone BAC 99M22	0
A26S_Cmac_wdnm_contig_20104	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	8.76E-16
A26S_Cmac_wdnm_contig_20107	*Calliphora vicina* Achaete-Scute clone BAC 113H10	1.39E-23
A26S_Cmac_wdnm_contig_20178	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.49E-53
A26S_Cmac_wdnm_contig_20269	*Calliphora vicina* Achaete-Scute clone BAC 97L04	6.72E-07
A26S_Cmac_wdnm_contig_2039	*Plutella xylostella* uncharacterized LOC105396675 (LOC105396675) mRNA	0
A26S_Cmac_wdnm_contig_20414	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_20424	*Bactrocera cucurbitae* splicing arginine serine-rich 15 (LOC105218468) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_20435	N/A	
A26S_Cmac_wdnm_contig_20438	*Cochliomyia hominivorax* transformer (tra) complete cds	1.80E-27
A26S_Cmac_wdnm_contig_20446	*Musca domestica* mediator of RNA polymerase II transcription subunit 26 (LOC101898239) transcript variant mRNA	9.69E-78
A26S_Cmac_wdnm_contig_20452	N/A	
A26S_Cmac_wdnm_contig_20537	N/A	
A26S_Cmac_wdnm_contig_20702	*Lucilia cuprina* prophage major tail sheath (LOC111679331) mRNA	1.00E-114
A26S_Cmac_wdnm_contig_20881	N/A	
A26S_Cmac_wdnm_contig_20906	N/A	
A26S_Cmac_wdnm_contig_20913	*Onthophagus taurus* uncharacterized LOC111419563 (LOC111419563) transcript variant ncRNA	4.26E-45
A26S_Cmac_wdnm_contig_20926	N/A	
A26S_Cmac_wdnm_contig_2105	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_21096	*Calliphora vicina* Achaete-Scute clone BAC 99M22	8.40E-55
A26S_Cmac_wdnm_contig_21118	*Rhagoletis zephyria* carboxypeptidase D (LOC108377769) transcript variant mRNA	1.00E-70
A26S_Cmac_wdnm_contig_212	*Lucilia cuprina* dimethyl sulfoxide reductase -like (LOC111679813) mRNA	0
A26S_Cmac_wdnm_contig_21216	N/A	
A26S_Cmac_wdnm_contig_21240	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.05E-102
A26S_Cmac_wdnm_contig_21369	*Musca domestica* titin (LOC101887274) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_21384	N/A	
A26S_Cmac_wdnm_contig_21467	*Bactrocera latifrons* uncharacterized LOC108966469 (LOC108966469) transcript variant mRNA	5.92E-122
A26S_Cmac_wdnm_contig_21511	N/A	
A26S_Cmac_wdnm_contig_21573	*Drosophila mojavensis* uncharacterized protein (Dmoj\GI17415), mRNA	6.94E-06
A26S_Cmac_wdnm_contig_21613	*Bactrocera oleae* venom serine carboxypeptidase (LOC106616648) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_21624	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.24E-11
A26S_Cmac_wdnm_contig_21664	N/A	
A26S_Cmac_wdnm_contig_2170	*Lucilia cuprina* ascorbate-specific PTS system EIIC component-like (LOC111688246) mRNA	0
A26S_Cmac_wdnm_contig_2173	*Lucilia cuprina* choline trimethylamine-lyase-like (LOC111685019) mRNA	0

Table 4. continued

A26S_Cmac_wdnm_contig_21805	*Lucilia cuprina* NF-kappa-B inhibitor alpha-like (LOC111681456) transcript variant mRNA	8.54E-39
A26S_Cmac_wdnm_contig_2185	*Stomoxys calcitrans* homeobox aristaless (LOC106095556) transcript variant mRNA	4.94E-149
A26S_Cmac_wdnm_contig_21930	*Rhagoletis zephyria* piggyBac transposable element-derived 3-like (LOC108376162) mRNA	5.39E-67
A26S_Cmac_wdnm_contig_21947	*Bactrocera dorsalis* no-on-transient A-like (LOC105227487) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_21990	*Cochliomyia macellaria* transformer (tra) complete cds	1.36E-29
A26S_Cmac_wdnm_contig_22014	*Bactrocera cucurbitae* uncharacterized LOC105216970 (LOC105216970) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_22130	N/A	
A26S_Cmac_wdnm_contig_22174	N/A	
A26S_Cmac_wdnm_contig_2219	*Plutella xylostella* aldehyde dehydrogenase family 8 member A1-like (LOC105396730) mRNA	0
A26S_Cmac_wdnm_contig_22193	*Calliphora vicina* Achaete-Scute clone BAC 104L14	2.50E-10
A26S_Cmac_wdnm_contig_22197	*Onthophagus taurus* NAD(P) mitochondrial-like (LOC111414060) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_22222	*Bactrocera latifrons* uncharacterized LOC108967653 (LOC108967653) ncRNA	4.15E-30
A26S_Cmac_wdnm_contig_22299	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	3.61E-07
A26S_Cmac_wdnm_contig_22454	*Rhagoletis zephyria* uncharacterized LOC108359128 (LOC108359128) mRNA	1.07E-34
A26S_Cmac_wdnm_contig_2247	*Lucilia cuprina* lysine acetyltransferase Pka-like (LOC111677980) mRNA	0
A26S_Cmac_wdnm_contig_22471	*Culicoides sonorensis* genome scaffold: scaffold45	7.82E-14
A26S_Cmac_wdnm_contig_22536	*Lucilia cuprina* uncharacterized LOC111688960 (LOC111688960) mRNA	1.81E-09
A26S_Cmac_wdnm_contig_22556	*Calliphora vicina* Achaete-Scute clone BAC 113H10	1.49E-36
A26S_Cmac_wdnm_contig_22734	*Drosophila hydei* uncharacterized LOC111602837 (LOC111602837) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_22746	*Cochliomyia hominivorax* transformer (tra) complete cds	4.18E-30
A26S_Cmac_wdnm_contig_22760	*Calliphora vicina* Achaete-Scute clone BAC 104L14	1.90E-10
A26S_Cmac_wdnm_contig_22837	*Culicoides sonorensis* genome scaffold: scaffold29	3.05E-06
A26S_Cmac_wdnm_contig_22851	N/A	
A26S_Cmac_wdnm_contig_22858	*Calliphora vicina* Achaete-Scute clone BAC 99M22	8.33E-43

Table 4. continued

A26S_Cmac_wdnm_contig_22865	*Cochliomyia macellaria* transformer (tra) complete cds	8.96E-22
A26S_Cmac_wdnm_contig_2287	*Onthophagus taurus* NAD(P) mitochondrial-like (LOC111414060) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_22880	*Calliphora vicina* Achaete-Scute clone BAC 99M22	8.76E-41
A26S_Cmac_wdnm_contig_22881	*Bactrocera dorsalis* Coiled-coil domain-containing 28B (Cc28b) transcript variant mRNA	3.72E-105
A26S_Cmac_wdnm_contig_2291	*Calliphora vicina* Achaete-Scute clone BAC 99M22	0
A26S_Cmac_wdnm_contig_22993	*Cochliomyia macellaria* transformer (tra) complete cds	4.95E-46
A26S_Cmac_wdnm_contig_22996	N/A	
A26S_Cmac_wdnm_contig_23017	*Acyrthosiphon pisum* ribosomal RNA small subunit methyltransferase B-like (LOC107885553) mRNA	4.25E-11
A26S_Cmac_wdnm_contig_23071	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.31E-43
A26S_Cmac_wdnm_contig_2313	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.22E-14
A26S_Cmac_wdnm_contig_23207	*Cochliomyia macellaria* transformer (tra) complete cds	3.73E-34
A26S_Cmac_wdnm_contig_23282	N/A	
A26S_Cmac_wdnm_contig_23452	*Calliphora vicina* Achaete-Scute clone BAC 113H10	7.03E-33
A26S_Cmac_wdnm_contig_235	N/A	
A26S_Cmac_wdnm_contig_23564	*Eurytemora affinis* RING finger nhl-1-like (LOC111713802) transcript variant mRNA	2.12E-27
A26S_Cmac_wdnm_contig_2358	*Drosophila melanogaster* clone complete sequence	7.05E-52
A26S_Cmac_wdnm_contig_2360	*Plutella xylostella* uncharacterized LOC105396341 (LOC105396341) mRNA	0
A26S_Cmac_wdnm_contig_23627	N/A	
A26S_Cmac_wdnm_contig_23674	*Plutella xylostella* uncharacterized LOC105396341 (LOC105396341) mRNA	0
A26S_Cmac_wdnm_contig_23718	*Cochliomyia macellaria* transformer (tra) complete cds	1.12E-37
A26S_Cmac_wdnm_contig_23761	*Apis cerana* transcription elongation factor SPT6 (LOC107995922) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_23792	*Gryllus bimaculatus* GBcontig15223	3.71E-23
A26S_Cmac_wdnm_contig_23823	*Stomoxys calcitrans* uncharacterized LOC106085956 (LOC106085956) transcript variant ncRNA	3.09E-81
A26S_Cmac_wdnm_contig_23941	*Cochliomyia macellaria* transformer (tra) complete cds	8.14E-33
A26S_Cmac_wdnm_contig_24085	*Bactrocera latifrons* sodium-dependent neutral amino acid transporter B(0)AT3 (LOC108971140) transcript variant mRNA	0

Table 4. continued

A26S_Cmac_wdnm_contig_2409	*Nasonia vitripennis* ATP-dependent RNA helicase DHX8 (LOC100118513) transcript variant mRNA	1.54E-20
A26S_Cmac_wdnm_contig_24124	*Calliphora vicina* Achaete-Scute clone BAC 99M22	7.73E-108
A26S_Cmac_wdnm_contig_24158	N/A	
A26S_Cmac_wdnm_contig_24276	*Lucilia cuprina* cuticle 7 (LOC111676634) mRNA	4.58E-91
A26S_Cmac_wdnm_contig_24337	*Lucilia cuprina* structure-specific endonuclease subunit SLX4-like (LOC111689643) mRNA	1.84E-24
A26S_Cmac_wdnm_contig_24409	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.54E-144
A26S_Cmac_wdnm_contig_24435	*Lucilia cuprina* glutamate receptor kainate 5-like (LOC111681205) mRNA	2.88E-56
A26S_Cmac_wdnm_contig_24484	*Centruroides sculpturatus* iron-sulfur NUBPL-like (LOC111612727) transcript variant mRNA	3.20E-179
A26S_Cmac_wdnm_contig_24505	*Calliphora vicina* Achaete-Scute clone BAC 97L04	7.59E-43
A26S_Cmac_wdnm_contig_24546	*Musca domestica* dnaJ homolog subfamily C member 8 (LOC101891770) transcript variant mRNA	4.29E-129
A26S_Cmac_wdnm_contig_24555	*Musca domestica* ankyrin repeat domain-containing 13D (LOC101892454) transcript variant mRNA	4.42E-81
A26S_Cmac_wdnm_contig_2457	*Lucilia cuprina* glutathione hydrolase proenzyme-like (LOC111688726) mRNA	0
A26S_Cmac_wdnm_contig_24724	N/A	
A26S_Cmac_wdnm_contig_24734	N/A	
A26S_Cmac_wdnm_contig_24767	*Cochliomyia macellaria* transformer (tra) complete cds	1.23E-30
A26S_Cmac_wdnm_contig_24780	*Calliphora vicina* Achaete-Scute clone BAC 97L04	6.73E-21
A26S_Cmac_wdnm_contig_2480	*Lucilia cuprina* FGGY carbohydrate kinase domain-containing -like (LOC111688242) mRNA	0
A26S_Cmac_wdnm_contig_2481	*Lucilia cuprina* PTS system EIIBC component (LOC111684865) mRNA	0
A26S_Cmac_wdnm_contig_24812	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_24884	*Drosophila melanogaster* clone complete sequence	3.81E-161
A26S_Cmac_wdnm_contig_24905	N/A	
A26S_Cmac_wdnm_contig_24934	*Bombyx mori* genomic chromosome BAC clone: complete sequence	4.80E-21
A26S_Cmac_wdnm_contig_24953	*Drosophila melanogaster* clone complete sequence	3.39E-60
A26S_Cmac_wdnm_contig_25032	*Calliphora vicina* Achaete-Scute clone BAC 99M22	6.90E-67
A26S_Cmac_wdnm_contig_25364	*Rhagoletis zephyria* uncharacterized LOC108359450 (LOC108359450) mRNA	1.31E-08

Table 4. continued

A26S_Cmac_wdnm_contig_255	*Lucilia cuprina* ribonuclease E-like (LOC111683192) mRNA	0
A26S_Cmac_wdnm_contig_25595	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.07E-11
A26S_Cmac_wdnm_contig_25630	*Bactrocera oleae* WD repeat-containing 81 (LOC106616760) transcript variant mRNA	7.54E-121
A26S_Cmac_wdnm_contig_25672	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.27E-37
A26S_Cmac_wdnm_contig_25735	*Drosophila melanogaster* clone complete sequence	5.00E-41
A26S_Cmac_wdnm_contig_258	*Lucilia cuprina* zinc cadmium lead-transporting P-type ATPase-like (LOC111675891) mRNA	0
A26S_Cmac_wdnm_contig_2585	*Plutella xylostella* sorting and assembly machinery component 50 homolog B-like (LOC105395821) mRNA	0
A26S_Cmac_wdnm_contig_25909	*Diuraphis noxia* general transcription factor II-I repeat domain-containing 2-like (LOC107169732) mRNA	8.02E-148
A26S_Cmac_wdnm_contig_25923	*Ceratitis capitata* WD repeat and FYVE domain-containing 3 (LOC101451655) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_26078	*Stomoxys calcitrans* quiver (LOC106094108) transcript variant mRNA	6.32E-14
A26S_Cmac_wdnm_contig_26239	N/A	
A26S_Cmac_wdnm_contig_26397	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	3.34E-47
A26S_Cmac_wdnm_contig_26420	*Ceratitis capitata* polyglutamine-repeat pqn-41 (LOC101462127) transcript variant mRNA	5.83E-48
A26S_Cmac_wdnm_contig_26452	*Ceratitis capitata* borderless (LOC101457583) transcript variant mRNA	6.57E-106
A26S_Cmac_wdnm_contig_26525	*Apis dorsata* tubulin gamma-1 chain-like (LOC102677740) mRNA	6.97E-07
A26S_Cmac_wdnm_contig_26601	*Lucilia cuprina* lectin subunit alpha-like (LOC111690460) mRNA	2.51E-59
A26S_Cmac_wdnm_contig_26668	*Bactrocera dorsalis* zinc finger 239 (LOC105225413) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_2672		
A26S_Cmac_wdnm_contig_2689	N/A	
A26S_Cmac_wdnm_contig_2690		
A26S_Cmac_wdnm_contig_2704	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_27100	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.99E-22
A26S_Cmac_wdnm_contig_27164	N/A	
A26S_Cmac_wdnm_contig_2719	*Stomoxys calcitrans* verprolin (LOC106083174) transcript variant mRNA	4.54E-83

Table 4. continued

A26S_Cmac_wdnm_contig_27415	*Bactrocera oleae* transcription factor grauzone-like (LOC106616086) transcript variant mRNA	2.49E-69
A26S_Cmac_wdnm_contig_2749	*Stomoxys calcitrans* uncharacterized LOC106087632 (LOC106087632) mRNA	2.31E-30
A26S_Cmac_wdnm_contig_2752	*Lucilia cuprina* chitinase (LOC111681974) mRNA	6.72E-42
A26S_Cmac_wdnm_contig_27550	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.30E-57
A26S_Cmac_wdnm_contig_276	*Cochliomyia macellaria* transformer (tra) complete cds	6.61E-10
A26S_Cmac_wdnm_contig_27735	*Drosophila miranda* strain MSH22 chromosome 4 clone complete sequence	1.49E-34
A26S_Cmac_wdnm_contig_2774	*Lucilia cuprina* 1-like (LOC111687747) mRNA	0
A26S_Cmac_wdnm_contig_27832	*Lucilia cuprina* zinc finger 846-like (LOC111685543) mRNA	1.47E-08
A26S_Cmac_wdnm_contig_27874	*Lucilia cuprina* SUN domain-containing 2-like (LOC111682586) transcript variant mRNA	3.14E-09
A26S_Cmac_wdnm_contig_27905	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.25E-22
A26S_Cmac_wdnm_contig_2793	*Calliphora vicina* Achaete-Scute clone BAC 99M22	4.25E-25
A26S_Cmac_wdnm_contig_27947	*Lucilia cuprina* cytochrome P450 307a1-like (LOC111686006) mRNA	2.16E-08
A26S_Cmac_wdnm_contig_27960	N/A	
A26S_Cmac_wdnm_contig_27968	*Cochliomyia macellaria* transformer (tra) complete cds	8.53E-32
A26S_Cmac_wdnm_contig_28181	*Drosophila melanogaster* strain rover (forR) chromosome 3R	1.06E-23
A26S_Cmac_wdnm_contig_28263	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.10E-18
A26S_Cmac_wdnm_contig_28278	N/A	
A26S_Cmac_wdnm_contig_2837	*Calliphora vicina* Achaete-Scute clone BAC 113H10	1.43E-26
A26S_Cmac_wdnm_contig_28373	*Cochliomyia macellaria* transformer (tra) complete cds	1.45E-70
A26S_Cmac_wdnm_contig_284	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_28470	N/A	
A26S_Cmac_wdnm_contig_2857	*Lucilia cuprina* outer membrane usher - like (LOC111674729) mRNA	0
A26S_Cmac_wdnm_contig_28581	*Cochliomyia macellaria* transformer (tra) complete cds	4.12E-42
A26S_Cmac_wdnm_contig_28592	N/A	
A26S_Cmac_wdnm_contig_28638	*Drosophila melanogaster* clone complete sequence	1.81E-20
A26S_Cmac_wdnm_contig_28768	*Calliphora vicina* Achaete-Scute clone BAC 99M22	0
A26S_Cmac_wdnm_contig_288	*Centruroides sculpturatus* probable cysteine--tRNA mitochondrial (LOC111630715) transcript variant mRNA	1.37E-148

Table 4. continued

A26S_Cmac_wdnm_contig_28913	*Calliphora vicina* Achaete-Scute clone BAC 62B24	7.17E-23
A26S_Cmac_wdnm_contig_28935	N/A	
A26S_Cmac_wdnm_contig_29080	N/A	
A26S_Cmac_wdnm_contig_2909		
A26S_Cmac_wdnm_contig_29120	N/A	
A26S_Cmac_wdnm_contig_29124	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.92E-21
A26S_Cmac_wdnm_contig_29255	*Calliphora vicina* Achaete-Scute clone BAC 99M22	2.22E-154
A26S_Cmac_wdnm_contig_29379	*Calliphora vicina* Achaete-Scute clone BAC 113H10	3.79E-16
A26S_Cmac_wdnm_contig_29457	N/A	
A26S_Cmac_wdnm_contig_29559	AF139718 *Chrysomya bezziana* peritrophin-48 complete cds	1.83E-08
A26S_Cmac_wdnm_contig_29678	*Lucilia cuprina* probable asparagine synthetase	0
A26S_Cmac_wdnm_contig_29784	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.43E-37
A26S_Cmac_wdnm_contig_29786	N/A	
A26S_Cmac_wdnm_contig_29931	*Stomoxys calcitrans* myotubularin-related 6 (LOC106085444) transcript variant mRNA	2.44E-163
A26S_Cmac_wdnm_contig_30009	N/A	
A26S_Cmac_wdnm_contig_3001	N/A	
A26S_Cmac_wdnm_contig_30019	*Lucilia cuprina* high affinity cationic amino acid transporter 1-like (LOC111675499) mRNA	2.62E-09
A26S_Cmac_wdnm_contig_3008	*Bombus impatiens* acetyl-coenzyme A synthetase (LOC100744587) transcript variant mRNA	2.95E-171
A26S_Cmac_wdnm_contig_30115	N/A	
A26S_Cmac_wdnm_contig_30118	*Calliphora vicina* Achaete-Scute clone BAC 99M22	6.65E-08
A26S_Cmac_wdnm_contig_30144	*Cochliomyia macellaria* transformer (tra) complete cds	8.69E-50
A26S_Cmac_wdnm_contig_30220	N/A	
A26S_Cmac_wdnm_contig_30283	N/A	
A26S_Cmac_wdnm_contig_30324	*Lucilia cuprina* uncharacterized LOC111688723 (LOC111688723) mRNA	7.83E-09
A26S_Cmac_wdnm_contig_30381	N/A	
A26S_Cmac_wdnm_contig_30388	*Lucilia cuprina* peptidase T-like (LOC111685821) mRNA	0
A26S_Cmac_wdnm_contig_304	*Lucilia cuprina* magnesium transporter -like (LOC111689025) mRNA	1.39E-140
A26S_Cmac_wdnm_contig_30573	*Rhagoletis zephyria* rho GTPase-activating 15 (LOC108378747) transcript variant mRNA	3.63E-57
A26S_Cmac_wdnm_contig_30596	*Calliphora vicina* Achaete-Scute clone BAC 97L04	2.60E-32
A26S_Cmac_wdnm_contig_30616	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.40E-19

Table 4. continued

A26S_Cmac_wdnm_contig_3062	*Lucilia cuprina* glycine oxidase-like (LOC111676314) mRNA	0
A26S_Cmac_wdnm_contig_30843	N/A	
A26S_Cmac_wdnm_contig_309	*Lucilia cuprina* dimethyl sulfoxide reductase -like (LOC111682967) mRNA	0
A26S_Cmac_wdnm_contig_3099	*Stomoxys calcitrans* juvenile hormone epoxide hydrolase 2 (LOC106085229) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_31034	N/A	
A26S_Cmac_wdnm_contig_31073	N/A	
A26S_Cmac_wdnm_contig_31092	*Pogonomyrmex barbatus* cleavage and polyadenylation specificity factor subunit 5 (LOC105432876) transcript variant mRNA	2.73E-99
A26S_Cmac_wdnm_contig_31117	*Bemisia tabaci* fuzzy homolog (LOC109033038) transcript variant misc_RNA	1.62E-11
A26S_Cmac_wdnm_contig_31189	*Lucilia cuprina* fasciclin-3 (LOC111691206) transcript variant mRNA	2.08E-118
A26S_Cmac_wdnm_contig_31211	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	5.64E-29
A26S_Cmac_wdnm_contig_31249	*Calliphora vicina* Achaete-Scute clone BAC 99M22	5.08E-103
A26S_Cmac_wdnm_contig_3126	*Stomoxys calcitrans* uncharacterized LOC106085956 (LOC106085956) transcript variant ncRNA	6.62E-17
A26S_Cmac_wdnm_contig_31298	*Calliphora vicina* Achaete-Scute clone BAC 62B24	3.97E-10
A26S_Cmac_wdnm_contig_313	*Lucilia cuprina* -like (LOC111678981) mRNA	0
A26S_Cmac_wdnm_contig_31341	*Ceratosolen solmsi* marchali biotin carboxylase chloroplastic-like (LOC105367013) mRNA	0
A26S_Cmac_wdnm_contig_31361	*Cochliomyia hominivorax* transformer (tra) complete cds	4.45E-13
A26S_Cmac_wdnm_contig_3141	N/A	
A26S_Cmac_wdnm_contig_31470	N/A	
A26S_Cmac_wdnm_contig_31533	*Drosophila buzzatii* Isis retrotransposon gag poly (gag) complete and pol poly (pol) cds	2.85E-81
A26S_Cmac_wdnm_contig_31600	*Cephus cinctus* sodium potassium calcium exchanger 3 (LOC107271678) transcript variant mRNA	3.00E-41
A26S_Cmac_wdnm_contig_31621	*Cochliomyia macellaria* transformer (tra) complete cds	3.19E-43
A26S_Cmac_wdnm_contig_31763	*Lucilia cuprina* HSP83 complete cds	1.16E-12
A26S_Cmac_wdnm_contig_31797	*Apis dorsata* autophagy-related 11-like (LOC102675602) mRNA	2.24E-23
A26S_Cmac_wdnm_contig_318	*Lucilia cuprina* lipopolysaccharide assembly B-like (LOC111682008) mRNA	1.29E-154
A26S_Cmac_wdnm_contig_31809	*Calliphora vicina* Achaete-Scute clone BAC 62B24	2.62E-25
A26S_Cmac_wdnm_contig_31861	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_3200	*Plutella xylostella* branched-chain-amino-acid aminotransferase chloroplastic-like (LOC105396549) mRNA	2.03E-175
A26S_Cmac_wdnm_contig_32071	N/A	
A26S_Cmac_wdnm_contig_32082	N/A	
A26S_Cmac_wdnm_contig_32117	N/A	
A26S_Cmac_wdnm_contig_32128	*Musca domestica* titin homolog (LOC101894949) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_3216	*Musca domestica* pro-resilin (LOC101893907) transcript variant mRNA	2.29E-24
A26S_Cmac_wdnm_contig_32386	*Rhagoletis zephyria* non-specific lipid-transfer (LOC108366409) transcript variant mRNA	7.49E-124
A26S_Cmac_wdnm_contig_3240	*Lucilia cuprina* HSP70 complete cds	3.39E-09
A26S_Cmac_wdnm_contig_32454	*Lucilia cuprina* 2-oxoglutarate dehydrogenase- mitochondrial (LOC111680032) mRNA	1.42E-26
A26S_Cmac_wdnm_contig_32462	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.41E-06
A26S_Cmac_wdnm_contig_32473	N/A	
A26S_Cmac_wdnm_contig_3269	*Lucilia cuprina* UPF0053 -like (LOC111677938) mRNA	0
A26S_Cmac_wdnm_contig_3270	*Lucilia cuprina* elongation factor G-like (LOC111678101) mRNA	0
A26S_Cmac_wdnm_contig_32706	N/A	
A26S_Cmac_wdnm_contig_32735	*Apis cerana* 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase classes I and II (LOC108001978) transcript variant mRNA	2.09E-07
A26S_Cmac_wdnm_contig_3279	*Plutella xylostella* uncharacterized LOC105395273 (LOC105395273) mRNA	0
A26S_Cmac_wdnm_contig_3280	*Plutella xylostella* chaperone chloroplastic-like (LOC105395264) mRNA	0
A26S_Cmac_wdnm_contig_32802	*Drosophila melanogaster* 211000022279479 sequence	1.64E-07
A26S_Cmac_wdnm_contig_32933	*Calliphora vicina* Achaete-Scute clone BAC 99M22	6.28E-108
A26S_Cmac_wdnm_contig_32961	N/A	
A26S_Cmac_wdnm_contig_33050	*Musca domestica* G1 S-specific cyclin-E (LOC101893514) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_33063	N/A	
A26S_Cmac_wdnm_contig_3313	*Lucilia cuprina* oxygen-dependent choline dehydrogenase-like (LOC111688641) mRNA	0
A26S_Cmac_wdnm_contig_33361	*Drosophila busckii* chromosome X sequence	1.20E-08
A26S_Cmac_wdnm_contig_33372	*Cochliomyia macellaria* transformer (tra) complete cds	1.16E-13

Table 4. continued

A26S_Cmac_wdnm_contig_33481	*Lucilia cuprina* NF-kappa-B inhibitor alpha-like (LOC111681456) transcript variant mRNA	1.30E-15
A26S_Cmac_wdnm_contig_3354	*Lucilia cuprina* 5-methylthioadenosine S-adenosylhomocysteine deaminase-like (LOC111677801) mRNA	0
A26S_Cmac_wdnm_contig_33542	N/A	
A26S_Cmac_wdnm_contig_33602	N/A	
A26S_Cmac_wdnm_contig_33618	*Drosophila bipectinata* uncharacterized LOC108129278 (LOC108129278) transcript variant mRNA	2.95E-56
A26S_Cmac_wdnm_contig_3363	N/A	
A26S_Cmac_wdnm_contig_33777	N/A	
A26S_Cmac_wdnm_contig_33801	N/A	
A26S_Cmac_wdnm_contig_33980	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.21E-06
A26S_Cmac_wdnm_contig_34058	*Lucilia cuprina* fas-associated death domain (LOC111686266) mRNA	9.00E-50
A26S_Cmac_wdnm_contig_34082	*Stomoxys calcitrans* pickpocket 28-like (LOC106085200) transcript variant mRNA	9.28E-64
A26S_Cmac_wdnm_contig_3414	*Calliphora vicina* Achaete-Scute clone BAC 99M22	8.09E-11
A26S_Cmac_wdnm_contig_34198	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	3.07E-60
A26S_Cmac_wdnm_contig_34391	*Lucilia cuprina* NF-kappa-B inhibitor alpha-like (LOC111681456) transcript variant mRNA	2.39E-33
A26S_Cmac_wdnm_contig_34709	*Cochliomyia macellaria* transformer (tra) complete cds	2.22E-08
A26S_Cmac_wdnm_contig_34736	*Lucilia cuprina* uncharacterized LOC111684175 (LOC111684175) mRNA	2.10E-59
A26S_Cmac_wdnm_contig_34785	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.67E-33
A26S_Cmac_wdnm_contig_3480	*Drosophila melanogaster* clone complete sequence	4.51E-105
A26S_Cmac_wdnm_contig_34886	N/A	
A26S_Cmac_wdnm_contig_34916	*Stomoxys calcitrans* fat-like cadherin-related tumor suppressor homolog (LOC106091366) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_34971	*Calliphora vicina* Achaete-Scute clone BAC 62B24	6.07E-06
A26S_Cmac_wdnm_contig_3503	*Lucilia cuprina* lactate 2-monooxygenase-like (LOC111679784) mRNA	0
A26S_Cmac_wdnm_contig_35077	N/A	
A26S_Cmac_wdnm_contig_35092	N/A	
A26S_Cmac_wdnm_contig_35219	*Cochliomyia macellaria* transformer (tra) complete cds	1.38E-22
A26S_Cmac_wdnm_contig_35313	*Ceratitis capitata* EH domain-binding 1 (LOC101456301) transcript variant mRNA	8.60E-78

Table 4. continued

A26S_Cmac_wdnm_contig_35388	*Musca domestica* mediator of RNA polymerase II transcription subunit 26 (LOC101898239) transcript variant mRNA	5.86E-34
A26S_Cmac_wdnm_contig_35396	N/A	
A26S_Cmac_wdnm_contig_354	*Lucilia cuprina* recombination-promoting nuclease pSLT051-like (LOC111689458) mRNA	0
A26S_Cmac_wdnm_contig_35518	*Calliphora vicina* Achaete-Scute clone BAC 99M22	5.90E-27
A26S_Cmac_wdnm_contig_35568	*Ceratitis capitata* uncharacterized LOC111591642 (LOC111591642) transcript variant mRNA	4.32E-35
A26S_Cmac_wdnm_contig_3564	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.94E-89
A26S_Cmac_wdnm_contig_35658	*Lucilia cuprina* uncharacterized LOC111681307 (LOC111681307) mRNA	1.93E-49
A26S_Cmac_wdnm_contig_3568	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.90E-65
A26S_Cmac_wdnm_contig_3602	*Bactrocera dorsalis* oxysterol-binding - related 9 (LOC105228878) transcript variant mRNA	1.71E-52
A26S_Cmac_wdnm_contig_36079	*Drosophila melanogaster* sequence scaffold complete sequence	4.55E-24
A26S_Cmac_wdnm_contig_36140	*Calliphora vicina* Achaete-Scute clone BAC 97L04	8.70E-29
A26S_Cmac_wdnm_contig_36188	N/A	
A26S_Cmac_wdnm_contig_36190	N/A	
A26S_Cmac_wdnm_contig_36271	N/A	
A26S_Cmac_wdnm_contig_36300	*Cochliomyia macellaria* transformer (tra) complete cds	8.98E-46
A26S_Cmac_wdnm_contig_36336	N/A	
A26S_Cmac_wdnm_contig_364	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_36417	*Calliphora vicina* Achaete-Scute clone BAC 113H10	4.37E-19
A26S_Cmac_wdnm_contig_36497	N/A	
A26S_Cmac_wdnm_contig_36500	*Varroa jacobsoni* uncharacterized LOC111263649 (LOC111263649) transcript variant ncRNA	6.32E-13
A26S_Cmac_wdnm_contig_36590	*Stomoxys calcitrans* type-1 angiotensin II receptor-associated (LOC106083013) transcript variant mRNA	9.71E-40
A26S_Cmac_wdnm_contig_36648	*Lucilia cuprina* probable small nuclear ribonucleo Sm D1 (LOC111688550) mRNA	8.29E-14
A26S_Cmac_wdnm_contig_36650	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	1.61E-53
A26S_Cmac_wdnm_contig_36726	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	5.27E-26
A26S_Cmac_wdnm_contig_36834	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.99E-29
A26S_Cmac_wdnm_contig_36888	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_36999	*Cochliomyia macellaria* transformer (tra) complete cds	5.16E-23
A26S_Cmac_wdnm_contig_37	*Nasonia vitripennis* 22 kDa relaxation - like (LOC107981719) mRNA	9.11E-89
A26S_Cmac_wdnm_contig_37049	*Calliphora vicina* Achaete-Scute clone BAC 113H10	8.09E-14
A26S_Cmac_wdnm_contig_37070	N/A	
A26S_Cmac_wdnm_contig_37073	*Lucilia cuprina* filamin-A (LOC111680403) transcript variant mRNA	4.41E-20
A26S_Cmac_wdnm_contig_37077	*Lucilia cuprina* MOB kinase activator-like 2 (LOC111677100) transcript variant mRNA	7.35E-31
A26S_Cmac_wdnm_contig_37085	*Lucilia cuprina* uncharacterized LOC111682587 (LOC111682587) transcript variant mRNA	3.77E-39
A26S_Cmac_wdnm_contig_3717	*Lucilia cuprina* dual specificity mitogen-activated kinase kinase 4 (LOC111674490) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_37178	*Lucilia cuprina* HSP24 complete cds	8.41E-22
A26S_Cmac_wdnm_contig_37233	*Rhagoletis zephyria* ribonuclease Oy (LOC108372592) transcript variant mRNA	1.20E-13
A26S_Cmac_wdnm_contig_37256	*Cochliomyia hominivorax* transformer (tra) complete cds	1.68E-07
A26S_Cmac_wdnm_contig_37262	N/A	
A26S_Cmac_wdnm_contig_37467	N/A	
A26S_Cmac_wdnm_contig_37483	N/A	
A26S_Cmac_wdnm_contig_37634	*Lucilia cuprina* uncharacterized LOC111688960 (LOC111688960) mRNA	5.68E-11
A26S_Cmac_wdnm_contig_37661	*Lucilia cuprina* secretory carrier-associated membrane 5 (LOC111687922) transcript variant mRNA	1.31E-77
A26S_Cmac_wdnm_contig_37729	N/A	
A26S_Cmac_wdnm_contig_37809	*Stomoxys calcitrans* RNA-binding squid (LOC106083465) transcript variant mRNA	1.93E-59
A26S_Cmac_wdnm_contig_37901	*Stomoxys calcitrans* Cep89 homolog (LOC106091322) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_37953	*Calliphora vicina* Achaete-Scute clone BAC 113H10	4.57E-07
A26S_Cmac_wdnm_contig_37971	*Calliphora vicina* Achaete-Scute clone BAC 99M22	2.54E-96
A26S_Cmac_wdnm_contig_3802	*Plutella xylostella* uncharacterized LOC105397023 (LOC105397023) mRNA	0
A26S_Cmac_wdnm_contig_38065	*Lucilia cuprina* uncharacterized LOC111675071 (LOC111675071) mRNA	3.76E-24
A26S_Cmac_wdnm_contig_38120	N/A	
A26S_Cmac_wdnm_contig_38189	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_38392	*Drosophila elegans* uncharacterized LOC108135871 (LOC108135871) ncRNA	1.19E-24
A26S_Cmac_wdnm_contig_38410	*Lucilia cuprina* neprilysin-4 (LOC111689263) mRNA	3.09E-97
A26S_Cmac_wdnm_contig_3845	*Lucilia cuprina* probable rhizopine catabolism regulatory (LOC111678136) mRNA	0
A26S_Cmac_wdnm_contig_38465	N/A	
A26S_Cmac_wdnm_contig_38508	*Calliphora vicina* Achaete-Scute clone BAC 113H10	5.51E-12
A26S_Cmac_wdnm_contig_38519	*Cochliomyia macellaria* transformer (tra) complete cds	4.18E-17
A26S_Cmac_wdnm_contig_38646	N/A	
A26S_Cmac_wdnm_contig_38667	*Cochliomyia macellaria* transformer (tra) complete cds	1.80E-11
A26S_Cmac_wdnm_contig_38684	*Lucilia cuprina* frizzled-4 (LOC111683791) mRNA	1.71E-11
A26S_Cmac_wdnm_contig_3874	N/A	
A26S_Cmac_wdnm_contig_38956	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.13E-12
A26S_Cmac_wdnm_contig_38976	*Cochliomyia macellaria* transformer (tra) complete cds	1.21E-16
A26S_Cmac_wdnm_contig_38989	*Musca domestica* homeobox 5 (LOC101894759) transcript variant mRNA	2.41E-43
A26S_Cmac_wdnm_contig_38993	N/A	
A26S_Cmac_wdnm_contig_39041	*Lucilia sericata* transformer (tra) complete cds	2.42E-13
A26S_Cmac_wdnm_contig_39051	*Lucilia cuprina* sex-determining region Y -like (LOC111680612) mRNA	5.67E-22
A26S_Cmac_wdnm_contig_39060	*Lucilia cuprina* high affinity cationic amino acid transporter 1-like (LOC111675499) mRNA	4.20E-10
A26S_Cmac_wdnm_contig_39089	N/A	
A26S_Cmac_wdnm_contig_39127	*Lucilia cuprina* structure-specific endonuclease subunit SLX4-like (LOC111689643) mRNA	1.46E-29
A26S_Cmac_wdnm_contig_39198	N/A	
A26S_Cmac_wdnm_contig_39369	*Lucilia cuprina* HSP83 complete cds	8.36E-17
A26S_Cmac_wdnm_contig_39417	*Bactrocera latifrons* methionine aminopeptidase 2 (LOC108974651) transcript variant mRNA	1.34E-141
A26S_Cmac_wdnm_contig_3944	*Lucilia cuprina* insertion element IS407 uncharacterized kDa -like (LOC111689672) mRNA	0
A26S_Cmac_wdnm_contig_3946	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_399	*Acyrthosiphon pisum* phenylacetaldehyde dehydrogenase-like (LOC107885388) mRNA	3.20E-14
A26S_Cmac_wdnm_contig_4072	*Culicoides sonorensis* genome scaffold: scaffold108	5.95E-07

Table 4. continued

A26S_Cmac_wdnm_contig_412	*Lucilia cuprina* DNA polymerase II-like (LOC111676655) mRNA	0
A26S_Cmac_wdnm_contig_4180	*Cochliomyia macellaria* transformer (tra) complete cds	1.20E-19
A26S_Cmac_wdnm_contig_4214	*Lucilia cuprina* periodic tryptophan 2 homolog (LOC111691207) mRNA	0
A26S_Cmac_wdnm_contig_423	N/A	
A26S_Cmac_wdnm_contig_4239	*Drosophila melanogaster* clone complete sequence	3.82E-33
A26S_Cmac_wdnm_contig_4276	*Lucilia cuprina* aminodeoxychorismate synthase component 1-like (LOC111681627) mRNA	1.02E-130
A26S_Cmac_wdnm_contig_4302	*Lucilia cuprina* uncharacterized mitochondrial g00820-like (LOC111690096) mRNA	2.71E-92
A26S_Cmac_wdnm_contig_4356	*Lucilia cuprina* magnesium transporter -like (LOC111683547) mRNA	0
A26S_Cmac_wdnm_contig_4371	*Calliphora vicina* Achaete-Scute clone BAC 62B24	9.35E-14
A26S_Cmac_wdnm_contig_4391	N/A	
A26S_Cmac_wdnm_contig_4394	N/A	
A26S_Cmac_wdnm_contig_4418	*Drosophila subobscura* map 12A chromosomal inversion A2 P275 region genomic sequence	1.27E-29
A26S_Cmac_wdnm_contig_4471	N/A	
A26S_Cmac_wdnm_contig_4489	*Tetranychus urticae* pyruvate kinase PKM-like (LOC107363200) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_4492	*Lucilia cuprina* DNA-directed RNA polymerase subunit beta (LOC111685820) mRNA	0
A26S_Cmac_wdnm_contig_4505	*Rhagoletis zephyria* p-aminobenzoyl-glutamate hydrolase subunit B-like (LOC108356048) mRNA	2.58E-129
A26S_Cmac_wdnm_contig_4630	*Lucilia cuprina* 2-hydroxy-1,4-benzoquinone reductase-like (LOC111674749) mRNA	1.15E-66
A26S_Cmac_wdnm_contig_4637	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	1.36E-18
A26S_Cmac_wdnm_contig_464	*Lucilia cuprina* probable outer membrane usher (LOC111688664) mRNA	0
A26S_Cmac_wdnm_contig_4704	*Lucilia cuprina* oxygen-dependent choline dehydrogenase-like (LOC111688641) mRNA	0
A26S_Cmac_wdnm_contig_4706	*Crataraea suturalis* genome	1.71E-11
A26S_Cmac_wdnm_contig_4788	N/A	
A26S_Cmac_wdnm_contig_479	N/A	
A26S_Cmac_wdnm_contig_4869	*Rhagoletis zephyria* RNA-directed DNA polymerase from mobile element jockey-like (LOC108363750) transcript variant mRNA	5.30E-64
A26S_Cmac_wdnm_contig_4898	N/A	

Table 4. continued

A26S_Cmac_wdnm_contig_4924	*Drosophila melanogaster* clone complete sequence	0
A26S_Cmac_wdnm_contig_4925	*Lucilia cuprina* uncharacterized LOC111677489 (LOC111677489) mRNA	2.32E-48
A26S_Cmac_wdnm_contig_4935	*Rhagoletis zephyria* uncharacterized LOC108373869 (LOC108373869) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_4949	N/A	
A26S_Cmac_wdnm_contig_5002	N/A	
A26S_Cmac_wdnm_contig_5034	*Stomoxys calcitrans* uncharacterized LOC106085319 (LOC106085319) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_5040	N/A	
A26S_Cmac_wdnm_contig_510	*Lucilia cuprina* HSP83 complete cds	1.13E-19
A26S_Cmac_wdnm_contig_5105	*Lucilia cuprina* glutathione hydrolase proenzyme-like (LOC111688726) mRNA	0
A26S_Cmac_wdnm_contig_5114	*Drosophila melanogaster* clone complete sequence	5.67E-179
A26S_Cmac_wdnm_contig_5183	N/A	
A26S_Cmac_wdnm_contig_5224	*Lucilia cuprina* 60S ribosomal L10a-2 (LOC111684017) mRNA	0
A26S_Cmac_wdnm_contig_5249	*Litopenaeus vannamei* ryanodine receptor cds	1.45E-10
A26S_Cmac_wdnm_contig_5281	*Cochliomyia macellaria* transformer (tra) complete cds	9.87E-19
A26S_Cmac_wdnm_contig_5303	*Bactrocera oleae* ribonucleoside-diphosphate reductase large subunit (LOC106627109) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_5331	*Calliphora vicina* Achaete-Scute clone BAC 97L04	4.42E-08
A26S_Cmac_wdnm_contig_5388	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.84E-38
A26S_Cmac_wdnm_contig_546	*Lucilia cuprina* peptide transport periplasmic -like (LOC111678720) mRNA	0
A26S_Cmac_wdnm_contig_5505	N/A	
A26S_Cmac_wdnm_contig_5549	*Calliphora vicina* Achaete-Scute clone BAC 62B24	3.86E-10
A26S_Cmac_wdnm_contig_5652	*Calliphora vicina* Achaete-Scute clone BAC 62B24	1.38E-08
A26S_Cmac_wdnm_contig_5672	N/A	
A26S_Cmac_wdnm_contig_568	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_5692	*Cochliomyia macellaria* transformer (tra) complete cds	1.68E-06
A26S_Cmac_wdnm_contig_5771	*Lucilia cuprina* ribokinase-like (LOC111681734) mRNA	0
A26S_Cmac_wdnm_contig_5788	*Drosophila virilis* gene for acid complete strain: Acph- country: Japan:Horioka	1.18E-25
A26S_Cmac_wdnm_contig_5799	DVU49102 *Drosophila virilis* transposon Penelope ORF1 complete cds	1.22E-69

Table 4. continued

A26S_Cmac_wdnm_contig_5904	*Cochliomyia macellaria* transformer (tra) complete cds	1.09E-15
A26S_Cmac_wdnm_contig_5909	N/A	
A26S_Cmac_wdnm_contig_5923	*Lucilia cuprina* ribonuclease E-like (LOC111688054) mRNA	0
A26S_Cmac_wdnm_contig_5947	*Lucilia cuprina* uncharacterized LOC111683043 (LOC111683043) mRNA	6.46E-57
A26S_Cmac_wdnm_contig_5950	*Cochliomyia macellaria* transformer (tra) complete cds	3.82E-29
A26S_Cmac_wdnm_contig_5959	N/A	
A26S_Cmac_wdnm_contig_5987	*Lucilia cuprina* alpha-D-glucose 1-phosphate phosphatase -like (LOC111678092) mRNA	1.52E-73
A26S_Cmac_wdnm_contig_604	*Lucilia cuprina* outer membrane usher -like (LOC111682055) mRNA	0
A26S_Cmac_wdnm_contig_6066	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	3.75E-10
A26S_Cmac_wdnm_contig_6067	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	2.00E-06
A26S_Cmac_wdnm_contig_6081	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.88E-57
A26S_Cmac_wdnm_contig_611	*Bombus impatiens* UDP-glucose 4-epimerase (LOC100746012) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_6137	*Lucilia cuprina* tRNA-(ms[2]io	0
A26S_Cmac_wdnm_contig_6166	*Lucilia cuprina* blue copper oxidase -like (LOC111685750) mRNA	0
A26S_Cmac_wdnm_contig_6182	*Calliphora vicina* Achaete-Scute clone BAC 99M22	9.17E-67
A26S_Cmac_wdnm_contig_6193	N/A	
A26S_Cmac_wdnm_contig_6287	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	7.84E-33
A26S_Cmac_wdnm_contig_6345	*Ceratitis capitata* BUB3-interacting and GLEBS motif-containing ZNF207 (LOC101458384) transcript variant misc_RNA	1.51E-59
A26S_Cmac_wdnm_contig_6378	*Cochliomyia macellaria* transformer (tra) complete cds	5.59E-39
A26S_Cmac_wdnm_contig_6392	N/A	
A26S_Cmac_wdnm_contig_6496	*Cochliomyia macellaria* transformer (tra) complete cds	1.05E-18
A26S_Cmac_wdnm_contig_6530	*Bactrocera oleae* 60 kDa heat shock mitochondrial (LOC106620015) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_6538	N/A	
A26S_Cmac_wdnm_contig_6547	*Lucilia cuprina* WD repeat-containing 81 (LOC111690331) mRNA	0
A26S_Cmac_wdnm_contig_6604	*Lucilia cuprina* 30S ribosomal S3 (LOC111689111) mRNA	0
A26S_Cmac_wdnm_contig_6610	*Musca domestica* focadhesin (LOC101899629) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_6644	*Lucilia cuprina* uncharacterized LOC111683171 (LOC111683171) mRNA	1.62E-15

Table 4. continued

A26S_Cmac_wdnm_contig_6685	*Lucilia cuprina* diaminopimelate decarboxylase-like (LOC111691190) mRNA	0
A26S_Cmac_wdnm_contig_6731	*Rhagoletis zephyria* uncharacterized LOC108358328 (LOC108358328) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_6789	*Drosophila biarmipes* zinc finger 2 (LOC108035982) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_6839	*Lucilia cuprina* disco-interacting 2 (LOC111684114) transcript variant mRNA	2.42E-49
A26S_Cmac_wdnm_contig_686	*Ceratosolen solmsi* marchali probable sulfate thiosulfate import ATP-binding (LOC105367029) mRNA	7.62E-30
A26S_Cmac_wdnm_contig_6952	*Culicoides sonorensis* genome scaffold: scaffold25	8.77E-06
A26S_Cmac_wdnm_contig_6960	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	4.64E-26
A26S_Cmac_wdnm_contig_6984	*Stomoxys calcitrans* tweety-2 (LOC106082620) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_706	*Plutella xylostella* heat shock cognate 90 kDa -like (LOC105397479) mRNA	0
A26S_Cmac_wdnm_contig_7066	*Lucilia cuprina* sex-determining region Y -like (LOC111680612) mRNA	3.29E-10
A26S_Cmac_wdnm_contig_7161	*Musca domestica* bcd gene for bicoid promoter exon 1 and joined CDS	2.68E-12
A26S_Cmac_wdnm_contig_7166	*Bombus terrestris* glutamine--fructose-6-phosphate aminotransferase	0
A26S_Cmac_wdnm_contig_7179	*Drosophila melanogaster* clone complete sequence	3.45E-108
A26S_Cmac_wdnm_contig_7189	*Musca domestica* uncharacterized LOC105262305 (LOC105262305) transcript variant mRNA	1.84E-07
A26S_Cmac_wdnm_contig_722	*Lucilia cuprina* tRNA-(ms[2]io	0
A26S_Cmac_wdnm_contig_7263		
A26S_Cmac_wdnm_contig_7301	*Calliphora vicina* Achaete-Scute clone BAC 99M22	4.40E-12
A26S_Cmac_wdnm_contig_7322	*Calliphora vicina* Achaete-Scute clone BAC 99M22	2.80E-46
A26S_Cmac_wdnm_contig_7400		
A26S_Cmac_wdnm_contig_7518	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.22E-15
A26S_Cmac_wdnm_contig_7536	*Calliphora vicina* Achaete-Scute clone BAC 97L04	5.93E-14
A26S_Cmac_wdnm_contig_7553	*Cochliomyia macellaria* transformer (tra) complete cds	5.18E-25
A26S_Cmac_wdnm_contig_7567	*Lucilia cuprina* uncharacterized LOC111686541 (LOC111686541) mRNA	1.55E-170
A26S_Cmac_wdnm_contig_760	N/A	
A26S_Cmac_wdnm_contig_767	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0

Table 4. continued

A26S_Cmac_wdnm_contig_7729	*Lucilia cuprina* structure-specific endonuclease subunit SLX4-like (LOC111689643) mRNA	1.26E-13
A26S_Cmac_wdnm_contig_7779		
A26S_Cmac_wdnm_contig_7799	N/A	
A26S_Cmac_wdnm_contig_7829	*Lucilia cuprina* proteoglycan 4 (LOC111676615) mRNA	9.91E-60
A26S_Cmac_wdnm_contig_7859		
A26S_Cmac_wdnm_contig_794	*Lucilia cuprina* inducible ornithine decarboxylase-like (LOC111682189) mRNA	0
A26S_Cmac_wdnm_contig_7966		
A26S_Cmac_wdnm_contig_7968	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.35E-42
A26S_Cmac_wdnm_contig_7972	*Lucilia cuprina* prophage antitermination Q homolog -like (LOC111685152) mRNA	5.78E-139
A26S_Cmac_wdnm_contig_8119	*Calliphora vicina* Achaete-Scute clone BAC 97L04	1.11E-101
A26S_Cmac_wdnm_contig_821	N/A	
A26S_Cmac_wdnm_contig_826	*Plutella xylostella* lon protease homolog peroxisomal-like (LOC105395409) mRNA	0
A26S_Cmac_wdnm_contig_8262	*Drosophila melanogaster* clone complete sequence	7.97E-71
A26S_Cmac_wdnm_contig_8405	*Calliphora vicina* Achaete-Scute clone BAC 99M22	1.41E-92
A26S_Cmac_wdnm_contig_8417	*Calliphora vicina* Achaete-Scute clone BAC 62B24	3.13E-10
A26S_Cmac_wdnm_contig_8471	*Drosophila busckii* disks large 1 tumor suppressor -like (LOC108606040) transcript variant mRNA	1.36E-15
A26S_Cmac_wdnm_contig_8479	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_8628	*Stomoxys calcitrans* uncharacterized LOC106082550 (LOC106082550) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_867	*Lucilia cuprina* motility B-like (LOC111684153) mRNA	0
A26S_Cmac_wdnm_contig_8693	*Rhagoletis zephyria* uncharacterized LOC108358328 (LOC108358328) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_8703	*Aedes aegypti* endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase (LOC5567412) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_8709	*Musca domestica* uncharacterized LOC109614039 (LOC109614039) transcript variant ncRNA	1.05E-06
A26S_Cmac_wdnm_contig_8749	*Lucilia cuprina* uncharacterized LOC111682213 (LOC111682213) mRNA	2.85E-28
A26S_Cmac_wdnm_contig_8769	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	7.81E-43

Table 4. continued

A26S_Cmac_wdnm_contig_8807	*Ceratitis capitata* uncharacterized LOC101456736 (LOC101456736) transcript variant mRNA	5.63E-168
A26S_Cmac_wdnm_contig_8815	*Lucilia cuprina* UDP-N-acetyl-D-mannosamine dehydrogenase-like (LOC111685115) mRNA	0
A26S_Cmac_wdnm_contig_8828	*Lucilia cuprina* conserved oligomeric Golgi complex subunit 4 (LOC111681424) mRNA	0
A26S_Cmac_wdnm_contig_8921	*Cochliomyia macellaria* transformer (tra) complete cds	4.54E-16
A26S_Cmac_wdnm_contig_8938	*Cochliomyia macellaria* transformer (tra) complete cds	2.18E-07
A26S_Cmac_wdnm_contig_8975	*Apis dorsata* 2-amino-3-ketobutyrate coenzyme A mitochondrial-like (LOC102679518) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_8995	N/A	
A26S_Cmac_wdnm_contig_9038	*Calliphora vicina* Achaete-Scute clone BAC 104L14	5.73E-10
A26S_Cmac_wdnm_contig_9078	N/A	
A26S_Cmac_wdnm_contig_908	*Plutella xylostella* succinyl- ligase	0
A26S_Cmac_wdnm_contig_9133	N/A	
A26S_Cmac_wdnm_contig_9226	N/A	
A26S_Cmac_wdnm_contig_9333	*Rhagoletis zephyria* uncharacterized LOC108358328 (LOC108358328) transcript variant mRNA	3.89E-43
A26S_Cmac_wdnm_contig_9415	N/A	
A26S_Cmac_wdnm_contig_9436	mucin-5AC (LOC108660358) transcript variant mRNA	0
A26S_Cmac_wdnm_contig_948	N/A	
A26S_Cmac_wdnm_contig_954	SITRRNA Endosymbiont *Sitophilus zeamais* 16S ribosomal complete	0
A26S_Cmac_wdnm_contig_958	N/A	
A26S_Cmac_wdnm_contig_9601	N/A	
A26S_Cmac_wdnm_contig_9647	N/A	
A26S_Cmac_wdnm_contig_9654	*Lucilia cuprina* HSP83 complete cds	7.28E-11
A26S_Cmac_wdnm_contig_971	*Culex pipiens* clone *Culex pipiens* quinquefasciatus- complete sequence	0
A26S_Cmac_wdnm_contig_9779	*Calliphora vicina* Achaete-Scute clone BAC 113H10	2.73E-13
A26S_Cmac_wdnm_contig_9797	*Drosophila virilis* strain 9 histone H4 and histone H2A cds	2.65E-25
A26S_Cmac_wdnm_contig_9821	*Lucilia cuprina* farnesol dehydrogenase-like (LOC111686940) mRNA	1.32E-166
A26S_Cmac_wdnm_contig_9832	*Lucilia cuprina* strain Tara CYP12A7 (cyp12a7) complete cds	1.28E-19
A26S_Cmac_wdnm_contig_9850	*Calliphora vicina* Achaete-Scute clone BAC 99M22	9.65E-72
A26S_Cmac_wdnm_contig_990	*Lucilia cuprina* XP55-like (LOC111682417) mRNA	0

Table 4. continued

A26S_Cmac_wdnm_contig_9928	*Drosophila melanogaster* Unmapped_Scaffold_8_D1580_D1567 sequence	5.76E-06
A26S_Cmac_wdnm_contig_994	*Cochliomyia macellaria* transformer (tra) complete cds	1.58E-24
A26S_Cmac_wdnm_contig_9943	*Lucilia cuprina* DNA-directed RNA polymerase subunit beta (LOC111685820) mRNA	4.94E-162